REMARKS

Entry of the foregoing, and further and favorable consideration of the claims, in light of the foregoing amendments and the following remarks, are respectfully requested.

By the present amendment, Applicants have deleted material from the specification and have canceled Claims 55-57 without prejudice or disclaimer, purely for the purpose of expediting prosecution. Applicants do not agree with the Examiner's assertions regarding this subject matter, and reserve the right to pursue the same in a continuation or divisional application.

Nonetheless, Applicants maintain that the above amendments to the specification should obviate the issues of priority, oath/declaration, drawings, specification and sequence disclosures. In addition, because Claims 55-57 have been canceled, the rejection of these claims under 35 U.S.C. §112, first paragraph is rendered moot.

The Examiner also rejected Claims 48-51, 53, 54, 56, 57, 59 and 60 as allegedly not being enabled for yeast alpha mating factors other than yeast alpha mating factor pre-sequence ending with arginine operably connected in translation reading frame to DNA encoding the mature human interferon alpha, and not other yeast alpha mating factor prepro sequences. This rejection is respectfully traversed.

The Examiner asserts that "[t]he 'protein' recited in the claims that is secreted or recovered has been assumed to be mature protein since the only protein recited in the claims is encoded by DNA that encodes a 'mature protein'". However, there is no such limitation in the claims currently pending. Indeed, the claims presently under consideration require only that the expression vehicle comprises "a promoter sequence for yeast alpha factor operably connected to a DNA sequence encoding a protein heterologous the yeast" which is transformed with that expression vehicle (Claims 47, 52-53, 58-60) or "a DNA sequence encoding a pre-pro peptide of yeast alpha factor operably connected in translation reading frame to a DNA sequence encoding a protein heterologous to the yeast" (Claims 48-51, 54). The phrase "operably connected" means only that the alpha factor sequences are

connected to the heterologous DNA in such a way that the DNA gets expressed in proper reading frame. Hence, the currently pending claims encompass the production of both mature, and incompletely processed protein. As such, the Examiner's discussion regarding incompletely processed protein is irrelevant to the currently pending claims. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 47-60 under 35 U.S.C. §102(e) and (g) as being allegedly anticipated by Brake et al U.S. Patent No. 4,870,008. This rejection is respectfully traversed.

The Brake '008 patent refers to an earliest filing date of January 12, 1983. In the *Singh v. Brake* interference to which the Examiner refers, Singh provided evidence which demonstrated that by October 1, 1982, Singh had obtained successful expression of a protein using a construct as recited in the claims (i.e., a DNA sequence encoding a pre-pro peptide of yeast alpha factor operably connected in translation reading frame to a DNA sequence encoding a protein heterologous to the yeast). However, that protein contained N-terminal Glu-Ala sequences. The Board did not agree that Singh obtained properly processed "mature" protein prior to the filing date of Brake, and as such, Brake prevailed in the interference. However, the present claims do not exclude the production of such Glu-Ala-containing proteins, and thus, Singh's October 1, 1982 production of incompletely processed heterologous protein is a reduction to practice which antedates the Brake '008 patent. Copies of the relevant documents and declarations from the interference are attached as an Appendix hereto. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 47-54 and 58-60 under 35 U.S.C. §102(e) as being allegedly anticipated by Kurjan et al. This rejection is respectfully traversed.

As the Examiner correctly noted, the present claims require that the yeast sequences are in proper translation reading frame to heterologous DNA. The Examiner alleges that Kurjan et al discloses yeast expression vehicles comprising a fusion of a segment of the

yeast alpha factor gene and heterologous DNA. However, the Examiner did not appreciate that Kurjan et al, which contains only prophetic examples, is not enabling for the production of heterologous proteins using yeast pre-pro sequences, because the methods described by Kurjan et al were not sufficient to produce heterologous proteins translated in the proper reading frame.

Submitted herewith is a Declaration by Dr. Ronald Hitzeman, which attests to these facts. Dr. Hitzeman agrees with statements made by Dr. Brake during the prosecution of U.S. Application Serial No. 06/487,950, filed April 25, 1983 to Barr et al. Specifically, Dr. Hitzeman notes the following:

> 7) In my professional opinion, the Kurjan et al. working examples 9a, 9b and 9c are not enabling, and would not teach one of ordinary skill in the art how to successfully accomplish fusion of the alpha-factor gene with a gene coding for a precursor of somatostatin, with a gene for corticotropin, or with a gene for a precursor-enkephalin, nor would the description in these examples, if followed, provide results which would prove the utility of the yeast alpha-factor signal sequence for producing secreted mature heterologous proteins. The technical basis for my opinion is the following discussion of examples 9a, 9b and 9c, pointing out defects which prevent these examples from being capable of achieving their stated purpose.

A. Defects in Example 9a, Kurjan et al.

(1) Treatment of the Kurjan et al. RH1 fragment to fill-in the HindIII cohesive ends (presumably by T4 DNA polymerase or Klenow enzyme) would result in filling-in both the HindIII and EcoRI ends, thus destroying the EcoRI site by not allowing it to be reformed by hybridization to a

complimentary sticky end. Kurjan et al., Example 9a at column 10, line 68 to column 11, line 5 says:

"The cohesive end of the HindIII site of this fragment is filled in enzymatically to produce a fragment denoted RH2 to be ligated to a segment of the somatostatin gene. The RH2 fragment is jointed to a PstI-EcoRI fragment (denoted PE) from the sequence that codes for presomatostatin (Goodman et al.)"

This step permanently destroys this EcoRI site at the same time as it fills in the HindIII site on the R1-2 fragment.

(2) The proposed fusion to the preprosomatostatin cDNA is made at a PstI site which has been modified by poly(dC), poly(dG) homopolymers, the exact length of which was not determined (Goodman et al., P.N.A.S. (1981) 77:5869). Thus, there is only a 1/3 chance of the resulting presomatostatin fusion being in-frame for translation of the correct amino acid sequence. In addition, the polyG sequence and the three 5' nucleotides (AAG) will result in additional residues [$(Gly)_n$-Lys] (where n is polyG nucleotides/3, GGG codes for Gly, AAG codes for Lys) between the alpha-factor leader and preprosomatostatin. Such residues can interfere with secretion of a fusion protein, particularly when additional charged amino acids, such as lysine, are present.

(3) Blunt-end ligation of the fragments provides no way of ensuring the proper orientation in the ligation products and the fragments will be able to form linear and circular concatamers from which the desired products cannot be released by EcoRI digestion (since the EcoRI sites were destroyed by the fill-in reaction in (1) above). Twelve different orientations are equally possible for the blunt end ligation taking two DNA segments at a time. When larger DNA ligation chains form the number of possible orientations becomes

extremely large. The blocked EcoRI site means that it cannot be used to liberate the desired products in order to screen for the correct sequence in order to clone the correct sequence and ensure production of the correct product.

(4) The PstI ends are 3' overhangs and thus cannot be filled in because of the specificity of T4 DNA polymerase or Klenow enzyme. The PstI ends must be rendered blunt by S1 nuclease digestion or by 3' exonuclease activity of T4 DNA polymerase or Klenow enzyme thus removing nucleotides. This is not discussed nor recognized by Kurjan et al.

(5) There are three PstI sites internal to the cDNA sequence, and thus a partial PstI digest must be performed to generate the desired PE fragment. A complete digestion which is presumed from the statement in Kurjan et al., column 11, lines 2-8, would destroy the desired "PE" DNA segment.

(6) Cleavage in the prosomatostatin sequence is at Arg-105, not Lys-105 as stated at line 28, column 11.

B. Defects in Example 9b by Kurjan et al.

(1) The same problems in using the blunt-end RH2 fragment to fuse to the SS fragment will occur as in example 9a, thereby creating a mixture of different species of product with additional amino acids at the amino terminal end.

(2) The 3' SmaI site in the SS fragment ends in the middle of an Arg codon, therefore there will not be a proper translational termination signal in

the fusion protein resulting in translational read-through. Translational read-through of this gene fusion results in the synthesis of a longer protein with an unknown C-terminal extension creating a fusion-protein other than ACTH alone. The extra amino acids might be removed by proteolytic processing as proposed, however it is likely that the additional amino acids would also interfere with proper secretion of the fusion protein and if not removed could destroy the physiological activity of the ACTH.

C. Defects in Example 9c by Kurjan et al.

(1) The problems introduced by blunt-end ligation of the fragments would be even more severe in this example, since at least three fragments are being ligated making the formation of improperly oriented DNA ligations the far majority. There are no measures described which ensure proper orientation of the fragments or which allow release of the desired ligation product from linear ligation products to enable cloning into the appropriate plasmid vector since the EcoRI site has been permanently destroyed by filling in enzymatically.

(2) Again, there is no translational stop codon introduced into the 3' fragment. Therefore, translational read-though will lead to the production of a fusion protein with an unknown C-terminal extension. Such extensions are known to interfere with proper secretion of the fusion protein and also change the character of the product produced from an enkephaline to a fusion peptide product containing an amino acid sequence of unknown length and composition which is not an enkephalin.

In light of the non-enabling nature of Kurjan et al for the production of heterologous proteins translated in proper reading frame, it is not an appropriate anticipating reference against the presently pending claims. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 47-54 and 58-60 as being allegedly anticipated under 35 U.S.C. §102(e) by Brake et al U.S. Patent No. 4,914,026. This rejection is respectfully traversed.

The Brake '026 patent refers to an earliest filing date of April 7, 1983. As such, the Brake '026 patent is also obviated by the evidence of reduction to practice of an embodiment within the scope of the present claims, prior to that date. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 55-57 under 35 U.S.C. §103 as being purportedly unpatentable over Kurjan et al in view of Goeddel et al. In light of the cancellation of Claims 55-57, this rejection is moot.

Further and favorable action in the form of a Notice of Allowance is believed to be in order, and is earnestly solicited.

If the Examiner has any questions regarding this amendment, or the application in general, she is encouraged to contact the undersigned directly so that prosecution may be expedited.

Respectfully submitted,
BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: June 8, 2004

By: Sharon E. Crane
Sharon E. Crane, Ph.D.
Registration No. 36,113

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Arjun SINGH	Group Art Unit: 1632
Application No.: 08/448,946	Examiner: S. Priebe
Filed: May 24, 1995	Confirmation No.: 1239
For: USE OF ALPHA FACTOR SEQUENCES IN YEAST EXPRESSION SYSTEMS	

DECLARATION OF RONALD A. HITZEMAN UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The undersigned, Ronald A. Hitzeman, does hereby declare and state that:

1) I make the following declaration based upon my knowledge and belief.

2) I received my Ph.D. in Biological Chemistry from the University of Michigan in 1977. Since that time, I have worked continuously on heterologous gene expression and secretion of heterologous (foreign to yeast) proteins by yeast. I have 38 publications in prestigious scientific books and journals and have 9 issued U.S. patents, as well as corresponding foreign patents which are listed on my curriculum vitae, attached.

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3) I directed the research at Genentech involving the isolation of the alpha factor gene (also independently isolated by Kurjan and Herskowitz) as well as a second alpha factor gene that they did not isolate.

4) My present employment is as a consultant for companies with yeast heterologous gene and heterologous protein secretion problems independently and through Genotypes, Inc. of which I am the president.

5) I have read and understand U.S. Patent No. 4,546,082 by Kurjan and Herskowitz from the Unversity of California in Berkeley, CA.

6) I agree with the following statements made by Anthony J. Brake in a declaration under 37 C.F.R. 1.132 signed 7/23/86 in U.S. Application Serial No. 06/487,950, filed April 25, 1983.

7) In my professional opinion, the Kurjan et al. working examples 9a, 9b and 9c are not enabling, and would not teach one of ordinary skill in the art how to successfully accomplish fusion of the alpha-factor gene with a gene coding for a precursor of somatostatin, with a gene for corticotropin, or with a gene for a precursor-enkephalin, nor would the description in these examples, if followed, provide results which would prove the utility of the yeast alpha-factor signal sequence for producing secreted mature heterologous proteins. The technical basis for my opinion is the following discussion of examples 9a, 9b and 9c, pointing out

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defects which prevent these examples from being capable of achieving their stated purpose.

A. Defects in Example 9a, Kurjan et al.

(1) Treatment of the Kurjan et al. RH1 fragment to fill-in the HindIII cohesive ends (presumably by T4 DNA polymerase or Klenow enzyme) would result in filling-in both the HindIII and EcoRI ends, thus destroying the EcoRI site by not allowing it to be reformed by hybridization to a complimentary sticky end. Kurjan et al., Example 9a at column 10, line 68 to column 11, line 5 says:

> "The cohesive end of the HindIII site of this fragment is filled in enzymatically to produce a fragment denoted RH2 to be ligated to a segment of the somatostatin gene. The RH2 fragment is jointed to a PstI-EcoRI fragment (denoted PE) from the sequence that codes for presomatostatin (Goodman et al.)"

This step permanently destroys this EcoRI site at the same time as it fills in the HindIII site on the R1-2 fragment.

(2) The proposed fusion to the preprosomatostatin cDNA is made at a PstI site which has been modified by poly(dC), poly(dG) homopolymers, the exact length of which was not determined (Goodman et al., P.N.A.S. (1981) 77:5869). Thus, there is only a 1/3 chance of the resulting presomatostatin fusion being in-frame for translation of the correct amino acid sequence. In addition, the polyG sequence and the three 5' nucleotides (AAG) will result in additional residues [$(Gly)_n$-Lys] (where n is polyG nucleotides/3, GGG codes for Gly, AAG codes for Lys) between the alpha-factor leader and preprosomatostatin. Such residues can interfere with secretion of

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a fusion protein, particularly when additional charged amino acids, such as lysine, are present.

(3) Blunt-end ligation of the fragments provides no way of ensuring the proper orientation in the ligation products and the fragments will be able to form linear and circular concatamers from which the desired products cannot be released by EcoRI digestion (since the EcoRI sites were destroyed by the fill-in reaction in (1) above). Twelve different orientations are equally possible for the blunt end ligation taking two DNA segments at a time. When larger DNA ligation chains form the number of possible orientations becomes extremely large. The blocked EcoRI site means that it cannot be used to liberate the desired products in order to screen for the correct sequence in order to clone the correct sequence and ensure production of the correct product.

(4) The PstI ends are 3' overhangs and thus cannot be filled in because of the specificity of T4 DNA polymerase or Klenow enzyme. The PstI ends must be rendered blunt by S1 nuclease digestion or by 3' exonuclease activity of T4 DNA polymerase or Klenow enzyme thus removing nucleotides. This is not discussed nor recognized by Kurjan et al.

(5) There are three PstI sites internal to the cDNA sequence, and thus a partial PstI digest must be performed to generate the desired PE fragment. A complete digestion which is presumed from the statement in Kurjan et al., column 11, lines 2-8, would destroy the desired "PE" DNA segment.

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(6) Cleavage in the prosomatostatin sequence is at Arg-105, not Lys-105 as stated at line 28, column 11.

B. Defects in Example 9b by Kurjan et al.

(1) The same problems in using the blunt-end RH2 fragment to fuse to the SS fragment will occur as in example 9a, thereby creating a mixture of different species of product with additional amino acids at the amino terminal end.

(2) The 3' SmaI site in the SS fragment ends in the middle of an Arg codon, therefore there will not be a proper translational termination signal in the fusion protein resulting in translational read-through. Translational read-through of this gene fusion results in the synthesis of a longer protein with an unknown C-terminal extension creating a fusion-protein other than ACTH alone. The extra amino acids might be removed by proteolytic processing as proposed, however it is likely that the additional amino acids would also interfere with proper secretion of the fusion protein and if not removed could destroy the physiological activity of the ACTH.

C. Defects in Example 9c by Kurjan et al.

(1) The problems introduced by blunt-end ligation of the fragments would be even more severe in this example, since at least three fragments are being ligated making the formation of improperly oriented DNA ligations the far majority. There are no measures described which ensure proper orientation of the fragments or which allow release of the desired ligation product from linear ligation products to

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enable cloning into the appropriate plasmid vector since the EcoRI site has been permanently destroyed by filling in enzymatically.

(2) Again, there is no translational stop codon introduced into the 3' fragment. Therefore, translational read-though will lead to the production of a fusion protein with an unknown C-terminal extension. Such extensions are known to interfere with proper secretion of the fusion protein and also change the character of the product produced from an enkephaline to a fusion peptide product containing an amino acid sequence of unknown length and composition which is not an enkephalin.

I, Ronald A. Hitzeman, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

6/2/04
Date

Ronald A. Hitzeman
Ronald A. Hitzeman
RAH

RESUME AND CURRICULUM VITAE

RONALD ARTHUR HITZEMAN, PH.D.

President of Genotypes, Inc.
Home and business address:
15 Banff Way
Pacifica, California 94044
Home phone: (650) 355-2405
Fax: (650) 359-3981

The initial focus of Genotypes, Inc. is to work for other companies to help them solve specific product production problems on a contractual basis. Examples of problems to solve have been yield improvement of specific gene products (proteins for pharmaceutical applications) by production strain (microorganisms) changes as well as product quality improvement by modification of transcriptional, translational, or posttranslational pathways. More complex alterations or additions to biochemical pathways in yeast have been performed. The microorganisms focused on are mainly the yeast, *Saccharomyces cerevisiae*, (some familiarity with the yeast, *Pichia pastoris*) and various bacteria. The word, genotypes, refers to the genetic pedigree or characteristics of a living organism. Our experimentation involves making changes at the DNA level (in chromosomes and plasmids) using a combination of biochemistry, molecular biology, and genetics to manipulate and improve production microorganisms for pharmaceutical and industrial applications. Improved protein production strains of *Saccharomyces cerevisiae* and improved expression plasmids are available from Genotypes for licensing.

PERSONAL

Date of Birth:	May 2, 1948
Citizenship:	United States
Marital Status:	Married; with 29 year-old son, M.D. from UCLA, resident at Sutter General Hospital in Sacramento.
Present Position:	President and Research Director of Genotypes, Inc., a consulting company located in the Bay Area (incorporated March 6th, 1992).

EDUCATION

Institution	Dates	Degree
Purdue University	9/66 - 6/70	B.S., Chemistry
The University of Michigan	9/71 - 5/73	M.S., Biochemistry
The University of Michigan	5/73 - 6/77	Ph.D., Biochemistry Supervisor: Dr. Alan R. Price
The University of California at Santa Barbara	7/77 - 5/80	Postdoctoral fellow Supervisor:Dr. John Carbon

ACADEMIC HONORS

Dean's List at Purdue University, 1966-1970
Academic Award for graduating within the top 10% at Purdue University, 1970

APPOINTMENTS

Teaching Fellow, Department of Biochemistry, The University of Michigan, 1971-1975, USPHS, NIH Training Grant 5 T01 GM00187-17.

Teaching Assignments:
Biochemistry for Nursing Students - fall 1972
Biochemistry for Dental Students - winter 1972
Biochemistry for Undergraduate and Graduate non-majors - fall 1973
Biochemistry Undergraduate Course - fall 1974

Graduate Student Research Assistant, The University of Michigan, 1975-1977, ERDA, Grant EY-76-S-02-2101.

Damon Runyon-Walter Winchell postdoctoral fellow in the laboratory of Dr. John Carbon at The University of California, 1977-1979.

Abbott Laboratories postdoctoral fellow in the laboratory of Dr. John Carbon at The University of California, August 1, 1979-May 31, 1980.

WORK EXPERIENCE:

2000-present

Consultant (as President of Genotypes) for separate companies helping with strain development, product development and improvement, and yield improvement:

1) Sequoia Biotech – two GenenExers involved with probiotics development:
   Frank Hagie and Dr. Glen Nedwin – Genotypes will run a lab to develop yeast and bacteria for probiotic delivery of protein products.
2) YPS (Yeast Protein Sciences)(for about 1 year) – production of pharmaceutical proteins in yeast, esp. human antibodies – Robert Leach headed this company – I have done some lab direction and bench work at Penn State University with a collaborator, Dr. Davis Ng. Company shut down 12/03 due to intellectual property problems.
3) Nastech – I have worked as a consultant for years and research reviewer in April, 2003. The Company is developing a platform technology for delivering both new small- and large molecular drugs by nasal administration. CEO is Dr. Steven Quay.
4) Patent work for Genentech
5) Genotypes solely owns US patent # 6,670,154 B1 (listed as #10 below, described reference 38) which describes the making of photosynthetic yeast for alcohol production. I am currently looking for someone to finance this work as well as to work on photosynthetic organisms to make alternative fuels.

March, 1992-2000

President of Genotypes, Inc., had a company lab located at 61 Airport Blvd. (Suite B) in South San Francisco with its mission to supply research lab work as well as consulting advice to client biotech companies. This was a natural expansion of the work I have been doing for many years. Some of the clients I have worked with in the past supported this effort as well as new clients. See 1st page for more extensive mission. Had up to 9 people under my supervision.

Jul.,1991-Oct., 1992

Consultant and researcher for Strohtech, a division of Stroh Brewery. Also worked for Strohtech (now Apex Bioscience, Inc.) through Genotypes.

Jan.,1991-Sept.,1992

Consultant and directed researchers for Amylin in San Diego- peptide product yield improvement and product modification enhancement.

July-Dec.,1990

Consultant for Phage (Pharmacia Genetic Engineering) and Kabi in San Diego. Ended due to shut down of facility by its owner company, Procordia, of Sweden. I was doing 1/2 time research at this facility and directing others.

Jan., 1990-Jan.,1993

Visiting Scientist (part-time) at University of California, Berkeley, Department of Biochemistry, in lab of Dr. Clinton Ballou. Further study of yeast secretion/glycosylation mutants that were isolated at Genentech as well as many new mutant strains isolated at Berkeley.

Oct., 1989-Oct., 1993

Consultant for many other companies.

October, 1989- Dec. 1990

Consultant, Kabi Peptide Hormones. Worked at Kabi (Stockholm,Sweden) - November and December, 1989 – EGF and IGF-1 peptide yield improvement from yeast.

October, 1989- present

Consultant for Genentech. Patent work ongoing.

June, 1980- Sept., 1989

Research Scientist at Genentech, Inc. (see publications 12-33, 36, and all patents except 4 and 8-11)

Dec., 1982- Sept., 1989

Senior Scientist, Department of Cell Genetics
Genentech, Inc.
460 Point San Bruno Boulevard
South San Francisco, CA

June,1981 - Dec., 1982
Scientist I, Department of Molecular Biology,
Genentech, Inc.

June 1980-June 1981
Scientist II, Department of Molecular Biology,
Genentech, Inc.

Management Experience:

I. I have managed from 2 to 9 people during my tenure as yeast lab director at Genentech (1980-Sept. 1989). Those reporting to me ranged from B.S. through Ph.D. degrees (postdoctoral fellows and more senior Ph.D.'s). I also was yeast project team leader for many years managing many different projects.

II. At the same time at Genentech, I also directed a media preparation laboratory for about 3 years with 3-4 people.

III. I have performed and managed research at Genotypes, managing from 4-9 people from 1992-2000. I also have directed the company as president and visited other companies to negotiate research contracts as well as to consult.

Some of My Research Collaborators:

1992- present	Partial list of clients (some confidential): Pfizer, Inc., Cephalon, Apex Bioscience, Khepri, Collagen Corp., Cohesion Technologies, Icos Corporation, Corvas International, Canji, Nastech, YPS, and Sequoia Biotech.	
1990 – 1993	Isolation and characterization of yeast mutants that affect glycosylation and golgi transport of secreted proteins Barker Hall.	Dr. Clinton Ballou Dept. of Biochemistry Barker Hall U.C. Berkeley
1988 - 1990	Production and Function of Mammalian Na^+/K^+ ATPase in Yeast	Dr. Robert Farley University of Southern California, School of Medicine
1987 - 1990	Growth Factor Secretion by Yeast	Kabi Peptide Hormones Dr. Par Gellerfors, Sweden
1983 - 1989	Human Serum Albumin Secretion by Yeast	Mitsubishi, Corp.
1985 - 1986	Human and Yeast Chimeric Phosphoglycerate Kinases (Gene and Protein Function)	Drs. Arthur Riggs and Maria Mas City of Hope, Los Angeles
1980 - 1981	Heterologous Gene Expression in Yeast	Drs. Benjamin Hall and Gustav Ammerer University of Washington

REFERENCES:

Dr. Richard A. Berg Vice President of Research and Development	Previously with Collagen Corp., and Cohesion Technologies. Now with FzioMed, Inc. 170-A Granada Drive San Luis Obispo, CA 93401 Phone: 805-546-0610 Fax: 805-546-0571 raberg@fzio.com
Dr. John Carbon (Postdoctoral Director) Professor Biological Sciences	University of California Department of Biological Sciences Santa Barbara, CA 93106
Dr. Maurille J. Fournier Professor Biological Chemistry	University of Massachusetts Department of Biochemistry Amherst, MA 01003
Dr. Dennis Henner Staff Scientist Vice President of Research	Was at Genentech, Inc 460 Point San Bruno Blvd. South San Francisco, CA 94080 Now at MPM Capital, home:650-359-2199
Mr. Joseph De Angelo Vice President of Research Apex Bioscience, Inc.	P.O. Box 12847 Research Triangle Park North Carolina 27709-2847 Phone: 919-405-4002 Email: jdeangelo@apexbioscience.com
Dr. Pat Gray Senior Director of Science	Was with ICOS Corporation 22021 20th Ave. S.E. Bothell, Washington 98021 Phone: 206-322-2286 206-650-6765

Ronald Hitzeman, Ph.D.

President and CEO of Genotypes, Inc. - founded Genotypes in 1992. Previously, Dr. Hitzeman was a senior scientist at Genentech, Inc. for approximately nine years during which time he was the first scientist (in collaboration with scientists from the University of Washington) to successfully express a heterologous protein, Leukocyte Interferon, in yeast. This led to a major patent, "Expression of Polypeptides in Yeasts". Dr. Hitzeman received his doctorate in Biochemistry from The University of Michigan in 1977 and was a postoctoral fellow from 1977-1980 at UC Santa Barbara under the supervision of Dr. John Carbon, a leading yeast researcher in Molecular Biology. Dr. Hitzeman's list of publications and patents are as follows:

PUBLICATIONS

1. Price, A.R., R. Hitzeman, J. Frato, and K. Lombardi. 1974. Rifampicin-Resistant Bacteriophage PBS2 Infection and RNA Polymerase in *Bacillus subtilis*. *Nucleic Acid Res.* 1: 1497-1502.

2. Hitzeman, R.A. 1978. DNA Polymerase Induced by *Bacillus subtilis* Bacteriophage PBS2. Ph.D., dissertation. University of Michigan.

3. Hitzeman, R.A., A.R. Price, J. Neuhard, and H. Mollgaard. 1978. Deoxyribonucleoside Triphosphates and DNA Polymerase in Bacteriophage PBS1-Infected *Bacillus subtilis*. In *DNA Synthesis; Present and Future* (Molineaux, I.J., and Kohiyama, M., eds.) Plenum Press, New York. pp. 255-266.

4. Hitzeman, R.A., A.N., Hanel, and A.R. Price. 1978. Dextran Sulfate as a Contaminant of DNA extracted from Concentrated Viruses and as an Inhibitor of DNA Polymerases. *J. Virol.* 27: 255-257.

5. Hitzeman, R.A., and A.R. Price. 1978. *Bacillus subtilis* Bacteriophage PBS2-Induced DNA Polymerase; Its Purification and Assay Characteristics. *J. Biol. Chem* 253: 8518-8525.

6. Hitzeman, R.A., and A.R. Price. 1978. Characterization of *Bacillus subtilis* Bacteriophage PBS2-Induced DNA Polymerase and Its Associated Exonuclease Activity. *J. Biol. Chem.* 253: 8526-8532.

7. Hitzeman, R.A. and A.R. Price. 1978. Relationship of *Bacillus subtilis* DNA Polymerase III to Bacteriophage PBS2-Induced DNA Polymerase and to the Replication of Uracil-Containing DNA. *J. Virol.* 28: 697-709.

8. Clarke, L., R. Hitzeman, and J. Carbon. 1979. Selection of Specific Clones from Colony Banks by Screening with Radioactive Antibody. *Methods Enzymol.*, 68: 436-442.

9. Hitzeman, R.A., A.C. Chinault, A.H. Kingsman, and J. Carbon. 1979. Detection of E. coli Clones Containing Specific Yeast Genes by Immunological Screening. In volume 14 of the series, ICN-UCLA Symposium on Molecular and Cellular Biology (Academic Press) pp. 57-68.

10. Hitzeman, R.A., L. Clarke, and J. Carbon. 1980. Isolation and Characterization of the Yeast 3-Phosphoglycerokinase Gene (PGK) by an Immunological Screening Technique. *J. Biol. Chem.* 255: 12073-12080.

11. Chalmers, J., M. Kuziora, R. Hitzeman, J. Carbon, and S. Wakil. 1983. Molecular Cloning of Fatty Acid Synthetase Genes from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 258: 11648-11653.

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Assignee: Genentech, Inc. (foreign patent documents also).

2. United States Patent #4,775,622, October 4, 1988. Submitted March 8, 1982. Expression, Processing, and Secretion of Heterologous Protein by Yeast. Inventors: R.A. Hitzeman and D.W. Leung. Assignee: Genentech, Inc. (foreign patent documents also).

3. Expression of Polypeptides in Yeast. Priority February 25, 1981. Inventors: R.A. Hitzeman, F.E. Hagie, B.D. Hall, and G. Ammerer. Applicants: Genentech, Inc. and the Board of Regents of the University of Washington. Patents are administered by WRF (Washington Research Foundation, http://www.wrfseattle.org/). US Patent Nos. 5,618,676, 5,854,018 5,856,123, and 5,919,651.

4. Novel Eukaryotic Vectors and Plasmids Having PGK Regulatory Signals. U.S. Patent Application Filed November 25, 1981.
Inventors: R.A. Hitzeman and J. Carbon. Assignee: U.C. Regents, a California Corporation (U.C. Case: 81-201-1). U.S. patent granted the end of 1989. US patent # 4,865,989.

5. Metallothionein Transcription Control Sequences and Use Thereof. Filing date: May 17, 1984. Inventors: Tina Etcheverry and Ronald Hitzeman. Assignee: Genentech, Inc. (Docket #100-244). US patent # 4,940,661 July 10, 1990.

6. Use of Yeast Homologous Signals to Secrete Heterologous Proteins. Filing date: April 25, 1983. Inventors: Chung Nan Chung, Mark Matteucci, and Ronald Hitzeman. Assignee: Genentech, Inc. (Docket #100-129). Other Patents (submitted for approval), United States Patent #5,010,003, April 23, 1991 (foreign patents also).

7. Yeast Strains for Increased Production and Secretion of Heterologous Proteins. Filing date: April 26, 1989.
Inventors: Robert Hamilton, Vanessa Chisholm, and Ronald Hitzeman. Assignee: Genentech, Inc. (Docket #6464).

8. Production of Recombinant Procollagen in Yeast. U.S. Patent Application Serial No. 08/546,047, filing date, 10/20/95. International patent application no. PCT/US96/16646, filing date 10/18/96. Inventors: D. Toman, R. Berg, G. Daniels, R. Hitzeman, and G. Chisholm, IV. Assignees: Collagen Corporation and Genotypes, Inc. WO97/14431. **Now US patent #6,472,171 on October 29, 2002.**

9. Novel Methods for the Production of Gelatin and Full-Length Triple Helical Collagen in Recombinant Cells. U.S. Patent Application Serial No. 60/084,828, filed May 8, 1998. (RECENTLY GRANTED, U.S. patent # 6,413,742, July 2, 2002 and Australian patent # 754608)
Inventors: R. Hitzeman, G. Chisholm, R. Chang, H. McMullin, and D. Olsen.
Assignees: Cohesion Technologies and Genotypes, Inc.

10. Novel Vectors and Methods for Transfer of Bacterial Genomes and Other DNAs into Eukaryotic Organisms to Add New Valuable Functions to the Eukaryotes. U.S. Patent Serial Application No. 60/094,294, filing date. 7/27/98.
Inventors: R. Hitzeman and G. Chisholm, IV. Assignee: Genotypes, Inc. US patent granted May 2003 – received notice of allowance. US patent # 6,670,154 B1, December 30, 2003.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Arjun SINGH	Group Art Unit: 1632
Application No.: 08/448,946	Examiner: S. Priebe
Filed: May 24, 1995	Confirmation No.: 1239
For: USE OF ALPHA FACTOR SEQUENCES IN YEAST EXPRESSION SYSTEMS	

SUBMISSION OF APPENDIX TO AMENDMENT DATED JUNE 8, 2004

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In further response to the Final Office Action dated October 8, 2003, attached is the Appendix referred to in the Amendment filed June 8, 2004.

If the Examiner has any questions regarding this amendment, or the application in general, she is encouraged to contact the undersigned directly so that prosecution may be expedited.

Respectfully submitted,
BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: June 28, 2004

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APPENDIX TO AMENDMENT DATED JUNE 8, 2004

TAB A	Singh v. Brake, 55 USPQ2d 1673 (Fed. Cir. 2000) (note p. 1674, 2nd column to p. 1675, line 2)
TAB B	Singh v. Brake, 65 USPQ2d 1641 (Fed. Cir. 2003) (note p. 1643, last paragraph of 1st column to 2nd column)
TAB C	Declaration of Arjun Singh
TAB D	Interferon assays dated August 2, 1982 – October 1, 1982
TAB E	Excerpts from Declaration of William Kohr
TAB F	Amino acid analysis dated October 1, 1982

U.S. Court of Appeals
Federal Circuit

Singh v. Brake

No. 99-1259

Decided August 4, 2000

PATENTS

1. Patentability/Validity — Date of Invention — Conception (§115.0403)

Issue of whether putative inventor's testimony has been sufficiently corroborated is determined by "rule of reason" analysis, in which all pertinent evidence is evaluated in order to reach sound determination of credibility of inventor's story; in present case, substantial evidence does not support finding by Board of Patent Appeals and Interferences that there was no evidence linking particular oligonucleotide ordered by junior party inventor with plan to design claimed DNA construct prior to senior party's application filing date, since structural/chemical characteristics of nucleotide were key to accomplishing successful loop deletion mutagenesis required to obtain claimed DNA construct, and since inventor's notation adjacent to DNA request form explicitly stated that nucleotide was to be used for accomplishing necessary loop deletion.

2. Patentability/Validity — Date of invention — Conception (§115.0403)

Rule that putative inventor's action in obtaining specific reagents with no "substantial use" other than to make claimed chemical compound constitutes evidence of significant corroborative value for purposes of reduction to practice applies with equal force in context of conception, especially if required reagents involve DNA molecules whose precise sequence is critical and unique to asserted conception; in present case, junior party inventor's action in ordering particular oligonucleotide had substantial corroborating value for inventor's claim to conception of DNA construct, since nucleotide had no "substantial use" other than to accomplish loop deletion mutagenesis necessary to obtain claimed construct.

3. Patentability/Validity — Date of invention — Conception (§115.0403)

Entries in inventor's laboratory notebook are entitled to corroborative weight even though they were not witnessed until several years after they were made, since under "rule of reason" analysis, fact that notebook entry has not been promptly witnessed does not necessarily disqualify it from corroborating conception, and in some cases conception may be proved solely on basis of subsequently-witnessed notebook entries.

Appeal from the U.S. Patent and Trademark Office, Board of Patent Appeals and Interferences.

Patent interference proceeding between Arjun Singh, junior party, and Anthony J. Brake, senior party. Junior party appeals from judgment awarding priority of invention to senior party. Vacated and remanded; Gajarsa, J., concurring in separate opinion.

Steven B. Kelber and Sharon E. Crane, of Long, Aldridge & Norman, Washington, D.C., for appellant.

Debra A. Shetka, Thomas E. Ciotti, and Catherine M. Polizzi, of Morrison & Foerster, Palo Alto, Calif.; Rachel Krevans, San Francisco, Calif.; Robert P. Blackburn and Joseph H. Guth, of Chiron Corp., Emeryville, Calif., for appellee.

Before Lourie, Schall, and Gajarsa, circuit judges.

Lourie, J.

Arjun Singh appeals from the judgment of the United States Patent and Trademark Office Board of Patent Appeals and Interferences awarding priority of invention to Anthony J. Brake. *See Singh v. Brake,* Paper No. 164 (BPAI May 11, 1998). Because certain of the Board's key findings underlying its conclusion that Singh failed to prove conception of the subject matter of the interference prior to the effective filing date of Brake were unsupported by substantial evidence, we vacate and remand. Because the Board did not address whether Brake's earliest application adequately described and enabled the disputed subject matter as required by 35 U.S.C. § 112, ¶ 1, we remand for determination of those issues as well.

BACKGROUND

Singh and Brake are parties to an interference consisting of a count corresponding to all thirty-seven claims of Brake's U.S. Patent 4,870,008 (hereinafter "Brake 2"), entitled "Secretory Expression in Eukaryotes," and claims 8 and 19-21 of Singh's application Ser. No. 07/552,719, entitled "Use of Alpha Factor Sequences in Yeast Expression Systems." The count, which is identical to claim 1 of Brake 2, reads as follows:

1. A DNA construct comprising a sequence of the following formula:

5'—L—S—Gene *—3',

where:

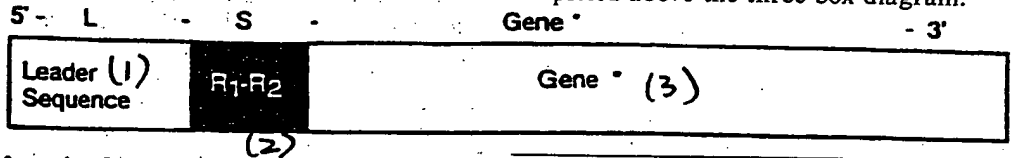
L encodes a Saccharomyces alpha-factor leader sequence recognized by a yeast host for secretion;

S encodes a spacer sequence providing processing signals resulting in the enzymatic processing by said yeast host of a precursor polypeptide encoded by L—S—Gene * into the polypeptide encoded by Gene *, S containing the sequence 5'—R_1—R_2—3' immediately adjacent to the sequence Gene *, R_1 being a codon for lysine or arginine, R_2 being [a] codon for arginine, with the proviso that S not contain the sequence 5'—R_3—R_4—X—3', where $R_3 = R_1$, $R_4 = R_2$, and X encodes a processing signal for dipeptidylaminopeptidase A; and

Gene * encodes a polypeptide foreign to Saccharomyces.

Paper No. 164 at 2-3.

As indicated by the count, the claimed DNA construct is comprised of three basic components: (1) DNA encoding an alpha-factor "leader sequence" (L) that directs a qc raul3eyeast cell to export the protein attached to it;[1] (2) a spacer (S) containing a first codon[2] R_1, that encodes lysine or arginine, followed by a second codon R_2, that encodes arginine; and (3) a gene (Gene *) that is foreign to yeast, that encodes a protein of interest. *See* '008 patent, col. 2, ll. 11-16, 38-43. The claimed DNA construct is illustrated in the figure below, with shorthand abbreviations of the three components depicted above the three-box diagram:



After the DNA construct has been introduced into the yeast cell, *e.g.*, via a plasmid vector, the cell translates the construct, yielding nascent protein ("protein construct"). The sequence of the protein construct, like the DNA encoding it, is divided into three regions: the 83-amino acid sequence of the alpha-factor leader, the Lysine-Arginine or Arginine-Arginine two-amino acid spacer, and the amino acid sequence of the protein of interest ("gene product").

The leader sequence functions to target the protein construct for secretion from the yeast cell. During secretion, the yeast enzyme KEX-2 recognizes the Lysine-Arginine or Arginine-Arginine spacer sequence in the protein construct and cleaves it at the junction between the spacer and the gene product. As a result, the desired gene product is released into the extracellular medium, free of the leader and spacer portions of the protein construct. *See* Paper No. 164 at 2. Because the yeast cell exports rather than retains the desired protein, protein purification is considerably simplified. *See id.*

In the course of Singh's attempts to design the claimed DNA construct in August 1982, he prepared plasmid p57, a circular DNA molecule containing the alpha-factor leader sequence and a spacer sequence directly adjacent to it.[3] *See* Singh Decl. ¶ 21. During that same month, Singh incorporated the gene for human protein interferon D ("IFN-D") into p57, thereby yielding plasmid p58. *See id.* In p58, the gene was also positioned adjacent to the spacer sequence, such that the leader, spacer, and gene sequences were all oriented in a fashion identical to the claimed construct. From September 6 to 11, 1982, Singh's assistant, Dr. June Lugovoy, isolated the DNA segment from p58 containing the alpha-factor leader, spacer, and IFN-D sequence, and inserted that segment (hereinafter "the p60 DNA construct") into yeast plasmid YEp9PT ("p60"). *See id.* ¶ 26. Plasmid p60 was then introduced into yeast cells to determine whether the p60 DNA construct would generate IFN-D. *See id.* ¶ 27.

On October 1, 1982, protein sequencing chemist Bill Kohr informed Singh that the IFN-D expressed by yeast cells transformed

[1] Alpha-factor, also known as alpha-mating factor, is a peptide released by the budding yeast *Saccharomyces cerevisiae* when a haploid cell is prepared to mate. *See* Bruce Alberts, et al., *Molecular Biology of the Cell* 722 (Garland Publishing, Inc. 3d ed. 1994). The yeast cell exports alpha-factor by way of a "leader sequence," which is attached to alpha-factor and signals that the peptide is to be exported from the cell. *See* Application Ser. No. 06/506,098, p. 3, ll. 3-5. That sequence is typically removed from alpha-factor upon secretion. *See id.* at p. 3, ll. 1-3. It is the alpha-factor leader sequence alone that is incorporated into the claimed construct.

[2] A "codon" is a set of three nucleotides that codes for a particular amino acid.

[3] The factual context of Singh's alleged conception of the claimed DNA construct is based on his declaration to the PTO and other record evidence. Absent qualification, the facts set forth here are not disputed by the parties.

with p60 contained eight additional amino acids not normally present in natural IFN-D. *See id.* ¶ 33. On approximately that same date, Singh alleges that he conceived the claimed DNA construct, *i.e.*, he devised a plan to redesign the p60 DNA construct in order to obtain the desired gene product, IFN-D, free of those additional amino acids. *See id.* ¶ 34. Specifically, Singh claims that he realized that he would need to remove eight unwanted codons (twenty-four nucleotides) from the p60 DNA construct, and that he planned to accomplish this deletion by use of a technique known as "loop deletion mutagenesis."[4]

On November 24, 1982, Singh wrote a laboratory notebook entry setting forth the undesired eight codons in the p60 DNA construct, as well as the twelve nucleotides on either side of that eight codon segment (the "flanking sequences"). *See* J.A. at 1380; Singh Decl. ¶ 45. On that date, Singh also ordered a linear, 24-nucleotide sequence (a "24-mer") that comprised the nucleotides of the flanking sequences. This order was canceled on the same day, and a notation in Singh's laboratory notebook stated that Singh would perform the deletion experiment in a different way "without changing codons." *Id.* On December 1, 1982, Singh ordered another 24-mer for the deletion experiment. This 24-mer was precisely complementary to the flanking sequences set forth in the November 24 entry. *See* J.A. at 1398; Singh Decl. ¶ 47. DNA chemist Peter Ng testified that he synthesized the 24-mer for Singh on December 20, 1982. *See* Ng Decl. ¶ 11; Ng Dep. at 36. Singh affixed the order into his notebook on December 21, 1982, with a notation "oligonucleotide for making in-frame deletion of pro-IFN-D junction." J.A. at 1398. Singh alleges that these facts corroborate his testimony that he conceived the claimed DNA construct before January 12, 1983, the filing date of Brake's application Ser. No. 06/457,325 (hereinafter "Brake 1").[5]

Based on their mutual claims to the DNA construct, an interference was declared between Singh and Brake. The parties filed an array of motions, only two of which are relevant here: Singh's motion to be accorded the benefit of the June 20, 1983 filing date of application Ser. No. 06/506,098, and Brake's motion to be accorded the benefit of the January 12, 1983 filing date of the Brake 1 application. The Administrative Patent Judge ("APJ") granted both motions, rendering Brake the senior party to the interference. *See* Paper No. 67 at 3-4.

Singh appealed to the Board, requesting reconsideration of the APJ's interlocutory decision that Brake should be accorded the benefit of the Brake 1 application. In its final decision, the Board concluded that the APJ did not abuse his discretion in granting Brake's motion,[6] but did not address Singh's contention that the Brake 1 application did not comply with the written description and enablement requirements of 35 U.S.C. § 112, ¶ 1 and hence that the APJ

[4] In "loop deletion mutagenesis," one removes undesired DNA sequences by first causing the undesired DNA to "bulge" (*i.e.*, form a loop) from the circular plasmid that contains it. Loop formation is accomplished by annealing a short, linear piece of "complementary" DNA to the sequences immediately flanking the undesired sequence. Following loop formation, the undesired DNA is excised by way of an appropriate enzyme that recognizes DNA loops and removes them.

A sequence of DNA is "complementary" to another sequence of DNA when the nucleotides comprising the two sequences follow normal base-pairing rules, i.e., every thymine in one sequence is aligned with a corresponding adenine in the other, and every guanine in one sequence is aligned with a corresponding cytosine in the other.

[5] The Brake 2 patent issued from application Ser. No. 07/081,302, filed August 3, 1987. The '302 application is a continuation of application Ser. No. 06/522,909, filed August 12, 1983. The '909 application is in turn a continuation-in-part of the Brake 1 application.

[6] At the time of the Board's decision, 37 C.F.R. § 1.655(a) directed the Board to review an APJ's interlocutory orders as follows:

> The Board may also consider whether entry of any interlocutory order was an abuse of discretion. All interlocutory orders shall be presumed to have been correct, and the burden of showing an abuse of discretion shall be on the party attacking the order.

37 C.F.R. § 1.655(a) (1998).

Because that version of section 1.655 suggested that the merits panel review *all* interlocutory orders for an abuse of discretion, the Patent and Trademark Office clarified that regulation to emphasize that only procedural rulings are to be reviewed under that standard, while merits rulings are to be reviewed without deference. *See* Consideration of Interlocutory Rulings at Final Hearings in Interference Proceedings, 64 Fed. Reg. 12900, 12901 (1999). The present version of 37 C.F.R. § 1.655(a) reads in relevant part as follows:

> The Board may also consider whether an interlocutory order should be modified. The burden of showing that an interlocutory order should be modified shall be on the party attacking the order. The abuse of discretion standard shall apply only to procedural matters.

37 C.F.R. § 1.655(a) (1999).

erred in granting Brake's motion.[1] *See* Paper No. 164 at 10-11. The Board then turned to Singh's alternative argument that even if the Brake 2 patent is accorded the benefit of the Brake 1 filing date, Singh still prevails because he conceived the claimed subject matter prior to January 12, 1983. After considering all of the evidence in support of Singh's alleged conception, the Board held that Singh failed to prove "that he had formulated a clear and complete picture in his mind of the invention within the scope of Count 1" prior to the Brake 1 filing date. *Id.* at 29. Accordingly, the Board awarded priority of the subject matter of the count to Brake, concluding that he was entitled to all thirty-seven claims of the Brake 2 patent. *See id.* at 30.

Singh appealed the Board's decision to this court. We have jurisdiction pursuant to 28 U.S.C. § 1295(a)(4)(A).

DISCUSSION

A. *Conception*

Singh argues that the Board erred in concluding that he failed to prove conception of the subject matter of the count prior to January 12, 1983, asserting that he provided sufficient corroboration of his testimony of conception under the "rule of reason." Specifically, Singh argues that the combination of the November 24 and December 21, 1982 notebook entries, the December 1, 1982 oligonucleotide order, and the testimony of DNA chemist Ng sufficiently corroborate his conception. Moreover, Singh contends that the fact that there was no use for the 24-mer ordered on December 1 other than to accomplish the desired loop deletion further corroborates his testimony.

Brake responds that the Board considered the totality of the evidence and correctly held that Singh did not prove that he had a definite, permanent idea of the claimed subject matter prior to the Brake 1 filing date. Specifically, Brake argues that Singh's evidence fails to corroborate his declaration under the "rule of reason" because none of Singh's evidence links the December 1 oligonucleotide order to a loop-deletion mutagenesis plan prior to January 12, 1983. Rather, Brake contends that Singh's evidence merely shows that Singh's approach for obtaining the claimed construct was in continual flux prior to January 12. Brake also asserts that Singh failed to prove that the December 1 oligonucleotide had no use other than to accomplish the loop deletion.

Conception is "the formation in the mind of the inventor[] of a definite and permanent idea of the complete and operative invention, as it is thereafter to be applied in practice." *Kridl v. McCormick*, 105 F.3d 1446, 1449, 41 USPQ2d 1686, 1689 (Fed. Cir. 1997) (internal quotation marks omitted). A conception must encompass all limitations of the claimed invention, *see id.*, and "is complete only when the idea is so clearly defined in the inventor's mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation," *Burroughs Wellcome Co. v. Barr Lab., Inc.*, 40 F.3d 1223, 1228, 32 USPQ2d 1915, 1919 (Fed. Cir. 1994).

[1] It is well-established that when a party seeks to prove conception via the oral testimony of a putative inventor, that party must proffer evidence corroborating that testimony. *See Mahurkar v. C.R. Bard, Inc.*, 79 F.3d 1572, 1577, 38 USPQ2d 1288, 1291 (Fed. Cir. 1996); *Price v. Symsek*, 988 F.2d 1187, 1194, 26 USQP2d 1031, 1036 (Fed. Cir. 1993). This rule addresses the concern that a party claiming inventorship might be tempted to describe his actions in an unjustifiably self-serving manner in order to obtain a patent or to maintain an existing patent. *See Kridl*, 105 F.3d at 1450, 41 USPQ2d at 1689 ("The tribunal must also bear in mind the purpose of corroboration, which is to prevent fraud, by providing independent confirmation of the inventor's testimony."); *Price*, 988 F.2d at 1194-95, 26 USQP2d at 1036-37; *cf. Eibel Process Co. v. Minnesota & Ont. Paper Co.*, 261 U.S. 45, 60 (1923). There is no particular formula that an inventor must follow in providing corroboration of his testimony of conception. *See Kridl*, 105 F.3d at 1450, 41 USPQ2d at 1689. Rather, whether a putative inventor's testimony has been sufficiently corroborated is determined by a "rule of reason" analysis, in which "[a]n evaluation of *all* pertinent evidence must be made so that a sound determination of the credibility of the inventor's story may be reached." *Price*, 988 F.2d at 1195, 26 USQP2d at 1037.

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[1] The Board stated as follows:
In view of our disposition of Singh's request, we need not consider the arguments presented on pp. 46-83 of the Singh Brief or pp. 13-39 of the Singh Reply Brief as to the deficiencies of the Brake 1 application. That is, Singh's arguments as to the failure of the Brake 1 application to satisfy the requirements of 35 U.S.C. § 112, first paragraph, are now moot.
Paper No. 164 at 10-11.

Conception is a question of law based on underlying facts. *See Eaton v. Evans,* 204 F.3d 1094, 1097, 53 USPQ2d 1696, 1698 (Fed. Cir. 2000). We review the Board's legal conclusion without formal deference, *see* 5 U.S.C. § 706 (1994), and its subsidiary factual findings for substantial evidence, *see In re Gartside,* 203 F.3d 1305, 1315, 53 USPQ2d 1769, 1775 (Fed. Cir. 2000).

After review of the record evidence in light of the proper legal standards, we conclude that substantial evidence does not support the Board's key finding that *no* evidence links the nucleotide Singh ordered on December 1, 1982 with a plan to design the claimed construct prior to January 12, 1983. Brake does not dispute that Singh recognized the problem of additional amino acids in the human IFN-D generated by the p60 DNA construct as of October 1, 1982. *See* Appellee's Br. at 41. As discussed previously, the p60 construct is similar to the claimed DNA construct, except that the p60 DNA construct contained eight unwanted codons. Accordingly, our inquiry focuses on Singh's asserted corroborating evidence as it relates to resolving the problem of removing the unwanted DNA from the p60 DNA construct. *See Burroughs,* 40 F.3d at 1229-30, 32 USPQ2d at 1921 ("The idea must be definite and permanent in the sense that it involves a specific approach to the particular problem at hand.").

As an initial matter, we conclude that the Board correctly held as a matter of law that Singh failed to prove that he conceived the claimed construct prior to December 1, 1982. In Singh's November 24, 1982 notebook entry, Singh articulated the problem to be solved with considerable specificity, noting: (1) the eight extraneous amino acids present in the IFN-D generated by the p60 DNA construct, (2) the twenty-four unwanted nucleotides that code for those amino acids, with a notation "sequence to be removed," and (3) the twelve nucleotides that are immediately upstream and the twelve nucleotides immediately downstream of that segment, *i.e.*, the flanking segments. *See* J.A. at 1380. We agree with Brake, however, that substantial evidence supports the Board's finding that this entry alone was insufficient to corroborate Singh's testimony, because while clearly articulating the problem, the entry did not provide the solution. *See* Paper No. 164 at 22-24. The Board's key findings in this regard, both of which are supported by substantial evidence in the notebook entry itself, are: that a linear 24-mer other than the one necessary to accomplish the deletion was first ordered,[8] and that the order was in any event canceled the same day, with a notation "will do in a different way and w/o changing codons." *Id.* at 23-24.

However, the Board's crucial finding that *no evidence* links the 24-mer that Singh ordered on December 1, 1982 with a plan to design the claimed construct by the loop deletion method prior to January 12, 1983 is unsupported by the evidence of record, and is in fact squarely contradicted by the evidence contained in Singh's December 21, 1982 notebook entry.[9] As we noted previously, that entry contains two crucial pieces of evidence: first, a "Synthetic DNA Request" form, dated December 1, 1982, in which Singh requested a 24-mer to carry out the loop deletion experiment, and second, a notation adjacent to the order explaining Singh's intended use for the 24-mer.

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[8] As noted above, rather than order the 24-mer complement of the sequences flanking the undesired sequence, Singh ordered a 24-mer *identical to* those sequences.

[9] As may be observed throughout the Board's decision, this crucial finding drove the Board's conclusion that Singh had not conceived the claimed construct prior to Brake. In its initial discussion of the corroborating value of the December 1 oligonucleotide order, the Board stated that:

> Nor do we find that Singh has provided any other evidence of his intended use of the oligonucleotide. That is, in neither the Brief, nor the Reply Brief does Dr. Singh point to any other evidence such as the disclosure of his invention to others, contemporaneous notebook entries, etc., which would link the nucleotide ordered on December 12 [sic, 1], 1982, with a plan to construct an invention within the scope of count 1.

Paper No. 164 at 25. The Board essentially reiterated this finding in the same discussion, remarking that:

> In our opinion, the only evidence of record which corroborates Dr. Singh's plan to employ the oligonucleotide ordered on December 1, 1982 in the "loop deletion," site-specific mutagenesis procedure as set forth in Figure 11 of Application 06/506,098 are the actual experiments performed by Dr. Singh on January 21, 1983, a date subsequent to the filing date of the Brake application.

Id. at 26. Finally, the Board echoed this finding in its concluding analysis, stating that:

> Nor do we find any evidence that those skilled in the art would have understood that the oligonucleotide ordered on December 1, 1982, was to be employed for the construction of a DNA construct in the loop deletion method described in Figure 11 of Singh Application 06/506,093, filed June 20, 1983.

Id. at 29.

As for the 24-mer, the Board makes no mention of the facts that the 24-mer is of *precisely the same length* and of the *precise complementarity* needed to accomplish the loop deletion, and thereby obtain the claimed construct; indeed, that oligonucleotide is one of 2.8 X 10^{14} possible 24-mers that Singh could have ordered. Because the structural/chemical characteristics of the 24-mer were key to accomplishing a successful loop deletion experiment, the order of that nucleotide, by itself, is evidence linking the 24-mer to Singh's plan to obtain the claimed construct. Moreover, the Board completely overlooked Singh's notation adjacent to the DNA request form that clearly specified that the 24-mer was to be used for accomplishing the necessary loop deletion. The notation explicitly stated that the 24-mer was ordered "for making in-frame deletion of pro-IFN-D junction." J.A. at 1398. Thus, both the properties of the 24-mer itself, and the notation specifying that it would be used to accomplish the loop deletion, undermine the Board's finding that *no evidence* links the 24-mer ordered on December 1 with Singh's plan to obtain the claimed construct by the loop deletion method prior to January 12, 1983.

[2] Moreover, the Board erred in rejecting Singh's argument that the 24-mer had corroborating value because it had no other "substantial use" than to accomplish the loop deletion. While the Board characterized that argument as "not the proper legal standard," Paper No. 164 at 25, that characterization conflicts with the holding of one of our predecessor courts, albeit in the context of reduction to practice, that when a putative inventor has obtained specific reagents with no "substantial use" other than to make the claimed chemical compound, that evidence is of significant corroborative value. *See Berges v. Gottstein*, 618 F.2d 771, 774-75, 205 USPQ 691, 694 (CCPA 1980). This rule applies with equal force in the context of conception, especially when the required reagents involve DNA molecules whose precise sequence is critical and unique to the asserted conception.

[3] In spite of those shortcomings in the Board's analysis, an argument could be made that the content of the notebook entries is entitled to no corroborative weight in any event because the entries were witnessed several years after they were made, thereby rendering those shortcomings harmless. While the witnessing of the laboratory notebooks fell far short of ideal, we do not agree that the belated witnessing undermines all corroborative value that these entries may possess. Under a "rule of reason" analysis, the fact that a notebook entry has not been promptly witnessed does not necessarily disqualify it in serving as corroboration of conception. *See Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1378, 231 USPQ 81, 89 (Fed. Cir. 1986) (holding that notebook entries not witnessed until several months to a year after entry did not render them "incredible or necessarily of little corroborative value" under the circumstances and in view of other corroborating evidence). Indeed, *Hybritech* indicates that in some cases, conception may be proved *solely* on the basis of laboratory notebook entries witnessed subsequent to their entry. *See id.* ("The laboratory notebooks, *alone*, are enough to show clear error in the findings that underlie the holding that the invention was not conceived before May 1980.") (emphasis added).

We also disagree with Brake's contention that *Hahn v. Wong* nullifies the corroborative value of the laboratory notebook entries. *See Hahn v. Wong*, 892 F.2d 1028, 1032, 13 USPQ2d 1313, 1317 (Fed. Cir. 1989) (stating that an inventor "must provide independent corroborating evidence in addition to his own statements and documents"). That case dealt with the standard of proof required to corroborate a reduction to practice, a more stringent standard than that required to corroborate a conception. *See Mikus v. Wachtel*, 542 F.2d 1157, 1161, 191 USPQ 571, 575 (CCPA 1976) (holding that an invention record, based on an unwitnessed laboratory notebook and results performed by technicians unaware of what they were testing, may provide sufficient evidence of conception but not reduction to practice under the rule of reason). Indeed, a notebook page may well show that the inventor *conceived* what he wrote on the page, whereas it may not show that the experiments were *actually performed*, as required for a reduction to practice. *Compare Hybritech*, 802 F.2d at 1378, 231 USPQ at 89 (indicating that an inventor's belatedly witnessed laboratory notebooks may alone be adequate to corroborate his testimony of conception) *with Gortatowsky v. Anwar*, 442 F.2d 970, 972, 170 USPQ 41, 43 (CCPA 1971) (holding that an inventor's laboratory notebook that was neither read nor witnessed and kept with suspect chronology could not provide the requisite corroboration for a reduction to practice).

In view of key facts contained in the December 21 notebook entry, we conclude that substantial evidence does not support the

Board's express finding that no evidence links the nucleotide Singh ordered on December 1 with a plan to design the claimed construct by the loop deletion method prior to January 12, 1983. We further conclude that the Board erred in rejecting Singh's argument that the 24-mer ordered on December 1 had corroborating value because it had no other "substantial use" than to obtain the claimed construct. We therefore vacate the Board's conception holding and remand for it to consider the evidence in the December 21 entry, to reconsider Singh's "substantial use" argument, and to reevaluate the totality of the corroborative evidence on remand. We have carefully considered Brake's other conception-related arguments but find them unpersuasive.

B. *Written Description and Enablement*

Singh also argues that the Board erred in concluding that Brake is entitled to the benefit of the filing date of Brake 1, asserting that the Brake 1 application fails to provide an adequate written description or enabling disclosure of the subject matter of the count. With regard to the written description requirement, Singh contends that Brake did not have possession of that subject matter, because Brake 1 describes a large genus of compounds and perhaps other species and subgenera, but not the particular subgenus (n = 0) within that genus.[10] Singh asserts that no "blazemarks" in Brake 1 lead a person of skill in the art to the n = 0 subgenus described in the count. *See In re Ruschig*, 379 F.2d 990, 994-95, 154 USPQ 118, 122 (CCPA 1967). As for enablement, Singh argues that Brake 1 does not enable the invention of the count, because it only leads a person of skill in the art to choose constructs wherein n > 0, and that, moreover, Brake provided no documentary evidence that techniques for making the n = 0 construct were available at the time of filing Brake 1. Lastly, Singh contends that the Board committed reversible error by applying the abuse of discretion standard rather than the *de novo* standard when reviewing the APJ's grant of Singh's motion for benefit.

Brake responds that the Board correctly held that he is entitled to the benefit of the filing date of Brake 1. On the issue of written description, Brake argues that Brake 1 explicitly describes the n = 0 construct, and that Singh's arguments were not raised below and are therefore waived. Even if we were to reach Singh's arguments, Brake asserts that there are two relevant permutations (n = 0 and n = 1 to 4), not 10,000, and that the requisite blazemarks are in fact present. Regarding enablement, Brake contends that Brake 1 does enable the n = 0 construct, and that Brake's enablement of "n = 1 to 4" does not indicate lack of enablement of n = 0. Brake also asserts that Singh's other enablement argument was not raised below and is in any event erroneous. Lastly, Brake argues that the appropriate standard of review was applied at the time of the Board's decision, and that even if the Board did apply the wrong standard, that error was harmless.

The Board explicitly did not reach any of the parties' section 112, paragraph 1 arguments, *see* note 7, *supra*, and we consider that such issues should be first decided by the Board. Accordingly, we remand to the Board for a determination of those issues that were properly raised during the earlier proceedings. Because no reliance interests by either party are impacted by the PTO's new procedural rule with respect to the Board's review of an APJ's interlocutory orders, we instruct the Board to apply the new standard, embodied in the current version of 37 C.F.R. § 1.655(a), on remand. *Cf. Landgraf v. USI Film Prods.*, 511 U.S. 244, 280 (1994) (stating that a new statute has retroactive effect if "it would impair the rights a party possessed when he acted, increase a party's liability for past conduct, or impose new duties with respect to transactions already completed") (cited in *Lowry v. Secretary of Health and Human Servs.*, 189 F.3d 1378, 1381 (Fed. Cir. 1999)).

[10] In his motion for benefit of the Brake 1 filing date, Brake relied on the following formula disclosed in Brake 1:

$$L\text{-}(R\text{-}S\text{-}(GAXYCX)_n)\text{-}Gene\ *)_y$$

where
L is a leader sequence
R is CGX or AZZ (a codon for Lysine or Arginine)
S is the same as R
X is any one of four nucleotides including T, G, C, or A
Y is either G or C
Z is either A or G
n is 0 or an integer that will typically vary from 1 to 4, usually 2 to 3
y is an integer of at least 1 and typically no greater than 10, and more typically not greater than 4

See Paper No. 15 at 6-7; '325 application, p. 3., l. 35 to p. 4, l. 26.

CONCLUSION

Certain of the Board's key findings underlying its conclusion that Singh failed to prove conception of the subject matter of the count prior to the effective filing date of Brake 2 are unsupported by substantial evidence. Moreover, the Board did not address issues relating to whether the Brake 1 application provides an adequate written description and an enabling disclosure of the subject matter of the count. We therefore

VACATE and *REMAND.*

Gajarsa, J., concurring.

I write separately in this case to emphasize that our decision today necessarily follows from our recent opinion in *In re Gartside,* 203 F.3d 1305, 53 USPQ2d 1769 (Fed. Cir. 2000). In *Gartside* this court held that, while we review questions of law *de novo*, the substantial evidence standard is appropriate for review of the PTO's factual findings. *See id.* at 1315, 53 USPQ2d at 1775. *Gartside* further explained that the pertinent inquiry under the substantial evidence standard is whether a reasonable factfinder could have arrived at the agency's decision, and that the inquiry requires "taking into account evidence that both justifies and detracts from the agency's decision." *Id.* at 1313, 53 USPQ2d at 1773 (quoting *Universal Camera Corp. v. National Labor Relations Bd.*, 340 US 474, 487-88 (1951)). The Supreme Court has also explained that the "possibility of drawing two inconsistent conclusions from the evidence does not prevent an administrative agency's finding from being supported by substantial evidence." *Consolo v. Federal Maritime Comm'n,* 383 U.S. 607, 620 (1966).

Under the Court's decision in *Dickinson v. Zurko,* 527 U.S. 150, 50 USPQ2d 1930 (1999), we must give proper deference to the PTO's factual determinations. Because of the deference that must be afforded, we cannot, and should not, ever substitute our own factual determinations for those made by the PTO. At the same time, when, as is the case here, the appellate tribunal finds that the PTO did not have substantial evidence to support its determinations, the proper course is to remand to have the PTO reweigh the sufficiency of the evidence in order to reach factual determinations that its expertise deems appropriate.

I offer one additional point. In its review of the Board's factual determination on corroboration, the majority opinion ignores evidence supporting Brake's position that was cited by the Board, while placing great emphasis on Singh's proffered evidence. This failure to distinguish the Board's supporting evidence runs dangerously close to turning the substantial evidence standard on its head. Perhaps this approach results from the less than clear recitation of the supporting evidence in the Board's decision. On remand, the Board should keep in mind our admonition in *Gechter v. Davidson*, 116 F.3d 1454, 1457, 43 USPQ2d 1040, 1043 (Fed. Cir. 1997) that "[n]ecessary findings must be expressed with sufficient particularity to enable our court, without resort to speculation, to understand the reasoning of the Board." *Cf.* 5 U.S.C. §557(c) (1994) (requiring that "[a]ll decisions" in formal adjudications, whether preliminary or final, "include . . . findings and conclusions, *and the reasons or basis therefor,* on all the material issues of fact, law, or discretion presented on the record." (emphasis added). A detailed account of the underlying evidence supporting a finding of fact, or the underlying facts supporting a conclusion of law, does more than exert discipline on the Board and expose its reasoning to the light of public scrutiny. It provides this court with a meaningful opportunity to determine whether the Board has strayed from the boundaries of its statutory authority. In this way, both the Board and this court benefit.

In this case, the Board on remand may ultimately reach the same factual determination on the issue of corroboration of Singh's testimony. Indeed, our decision today in no way forecloses this outcome. If so, a clear recitation of the evidence supporting this determination will only assist this court on appeal in its task of reviewing such a determination under the substantial evidence standard.

With this in mind, I concur in the judgment of the court.

U.S. District Court
Southern District of New York

Penguin Books U.S.A. Inc. v. New Christian Church of Full Endeavor Ltd.

No. 96 Civ. 4126 (RWS)

Singh v. Brake

U.S. Court of Appeals
Federal Circuit
No. 01-1621
Decided January 29, 2003

(Nonprecedential Opinion Issued October 16, 2002)

PATENTS

[1] Practice and procedure in Patent and Trademark Office — Interference — Rules and rules practice (§ 110.1704)

Practice and procedure in Patent and Trademark Office — Interference — Pleadings and submissions (§ 110.1706)

Board of Patent Appeals and Interferences did not abuse its discretion by returning junior party's brief, without further consideration, on ground that brief presented new arguments in derogation of board's reminder that only issues properly raised in earlier proceeding were entitled to review at final hearing, since junior party could have raised arguments in question at outset of interference, and to extent he did not do so, those arguments were waived, and since board's refusal to consider untimely arguments is not abuse of its discretion.

[2] Patentability/Validity — Date of invention — Conception (§ 115.0403)

Substantial evidence support's finding by Board of Patent Appeals and Interferences that entries in laboratory notebook fail to corroborate junior party inventor's claim to prior conception of DNA construct that is subject of interference count, since there is nothing in notebook to corroborate inventor's testimony that three entries in question were meant to be read together, since notebook entries, at most, state goal that inventor hoped to achieve, but they do not provide any protocol or outline for loop deletion mutagenesis procedure necessary to obtain claimed construct, and since there is no evidence to support inventor's assertion that loop deletion mutagenesis had been developed before his claimed date of conception, or that he knew of such developments prior to senior party's filing date.

[3] Practice and procedure in Patent and Trademark Office — Prosecution — Filing date (§ 110.0906)

Patentability/Validity — Specification — Written description (§ 115.1103)

Board of Patent Appeals and Interferences did not err in concluding that written description in senior party's earlier-filed application supports claim for DNA construct corresponding to interference count, since record does not support junior party's contention that specification in earlier application disclosed genus encompassing over 9000 species, of which count is directed to only two, or that specification failed to provide adequate direction to those of ordinary skill in art to lead them to subgenus of count, and since deference afforded board's conclusion under "substantial evidence" standard of review warrants finding of no legal error.

[4] Patentability/Validity — Date of invention — Conception (§ 115.0403)

Patentability/Validity — Specification — Enablement (§ 115.1105)

Finding by Board of Patent Appeals and Interferences that methods for obtaining DNA construct of interference count were available to those of ordinary skill in art as of filing date of senior party's earlier-filed application does not conflict with its conclusion that junior party inventor failed to establish prior conception of invention of count, since test for determining whether junior party conceived construct of count depends on his own personal knowledge of methods for making construct, and his formulation of definite and permanent idea for doing so, whereas test for whether senior party's application provided enabling disclosure does not depend on what senior party knew, but on whether application would have enabled one skilled in art to make and use invention at time application was filed.

Particular patents — Chemical — DNA constructs

4,870,008, Brake, secretory expression in eukaryotes, judgment awarding inventor priority of invention, with respect to all claims of patent, in interference no. 102,728 affirmed.

Appeal from the U.S. Patent and Trademark Office, Board of Patent Appeals and Interferences.

Patent interference proceeding no. 102,728 between Arjun Singh, junior party (application serial no. 07/552,719), and Anthony J. Brake (patent no. 4,870,008), senior party. Junior party appealed from judgment awarding priority of invention to senior party, which was reversed and remanded (55 USPQ2d 1673). On remand, board again awarded judgment to senior party, from which junior party now appeals. Affirmed.

Prior decision: 55 USPQ2d 1673.

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Before Lourie, circuit judge, Friedman, senior circuit judge, and Prost, circuit judge.

Lourie, J.

Arjun Singh appeals from the remand decision of the United States Patent and Trademark Office Board of Patent Appeals and Interferences awarding judgment in an interference to Anthony Brake. *Brake v. Singh*, Inter. No. 102,728, Paper No. 199 (Bd. Pat. App. & Inter. June 19, 2001). Because the Board's decision was supported by substantial evidence and was not contrary to law, we affirm.

BACKGROUND

This case arises out of an interference declared on November 12, 1991, involving a count corresponding to all thirty-seven claims of Brake's U.S. Patent 4,870,008 (hereinafter "the Brake patent") and claims 8 and 19-21 of Singh's U.S. Application 07/552,719.

The Brake patent issued from U.S. Application 07/081,302, filed August 3, 1987, which was a continuation of, and was accorded the benefit of, U.S. Application 06/522,909 (hereinafter "Brake 2"), filed August 12, 1983, assigned to Chiron Corporation. Singh's Application 07/552,719 was filed July 16, 1990, and was accorded the benefit of U.S. Application 06/506,098 (hereinafter "the Singh application"), filed June 20, 1983, and U.S. Application 06/488,323, filed April 25, 1983, both assigned to Genentech, Inc.

Because the earlier Singh application predated Brake 2, Singh was initially designated the senior party in the interference. However, Brake 2 was a continuation-in-part of U.S. Application 06/457,325 (hereinafter "Brake 1"), filed January 12, 1983, and Brake successfully moved for the benefit of the filing date of Brake 1 with respect to the count in the interference. Brake also successfully moved to attack the benefit accorded Singh of the April 25, 1983 filing date of U.S. Application 06/488,323. Brake was then designated as the senior party.

The count, which is identical to claim 1 of Brake 2, reads as follows:

> 1. A DNA construct comprising a sequence of the following formula:
>
> 5'-L-S-Gene*-3',
>
> where:
>
> L encodes a Saccharomyces alpha-factor leader sequence recognized by a yeast host for secretion;
>
> S encodes a spacer sequence providing processing signals resulting in the enzymatic processing by said yeast host of a precursor polypeptide encoded by L-S-Gene* into the polypeptide encoded by Gene*, S containing the sequence 5'-R_1-R_2-3' immediately adjacent to the sequence Gene*, R_1 being a codon for lysine or arginine, R_2 being codon for arginine, with the proviso that S not contain the sequence 5'-R_3-R_4-X-3', where R_3=R_1, R_4=R_2,
>
> and X encodes a processing signal for dipeptidylaminopeptidase A; and Gene* encodes a polypeptide foreign to Saccharomyces.

Brake, Paper No. 199 at 6.

The DNA construct of the count thus includes three basic components: (1) a segment, "L," which encodes an alpha-factor leader sequence;[1] (2) a segment, "S," which includes

[1] Alpha-factor, also known as alpha-mating factor, is a peptide released by the budding yeast *Saccharomyces cerevisiae* when a haploid cell is prepared to mate. *See* Bruce Alberts et al., *Molecular Biology of the Cell* 722 (3d ed. 1994). The yeast cell exports alpha-factor by way of a "leader sequence," which is attached to alpha-

a first codon,[2] R_1, encoding either lysine or arginine, followed by a second codon, R_2, encoding arginine; and (3) a gene, "Gene*," which encodes a protein of interest, in particular, a polypeptide foreign to (*i.e.*, not naturally produced by) the yeast *Saccharomyces*. *See* Brake patent, col. 2, ll. 11-16, 38-43.

After the DNA construct has been introduced into the yeast cell, *e.g.*, via a plasmid vector, the cell "expresses" the construct, producing a polypeptide having the sequence of amino acids encoded by the DNA. The sequence of the resulting polypeptide, like the DNA encoding it, is divided into three regions: the alpha-factor leader, the spacer sequence including either a lysine-arginine or an arginine-arginine two-amino acid block, and the amino acid sequence of the protein of interest ("gene product").

According to the record in this case, the leader sequence functions to target the polypeptide for secretion from the yeast cell. During secretion, the yeast enzyme KEX-2 recognizes the lysine-arginine or arginine-arginine spacer sequence in the polypeptide and cleaves the polypeptide at the junction between the spacer and the gene product. As a result, the desired gene product is released into the extracellular medium, free of the leader and spacer portions of the polypeptide. *See Brake*, Paper No. 164 at 2. Because the yeast cell exports rather than retains the desired protein, protein purification is considerably simplified. *See id.*

The following is a statement of the facts as set forth in our earlier opinion in this case. *Singh v. Brake*, 222 F.3d 1362, 55 USPQ2d 1673 (Fed. Cir. 2000). As we noted in that opinion, the factual context of Singh's alleged conception of the claimed DNA construct is based on his statements to the PTO and other record evidence. Absent qualification, the facts set forth here are not disputed by the parties.

In the course of Singh's attempts to design the claimed DNA construct in August 1982, he prepared plasmid p57, a circular DNA molecule containing the alpha-factor leader sequence and a spacer sequence directly adjacent to it. *See* Singh Decl. ¶ 21. During that same month, Singh incorporated the gene for human protein interferon D ("IFN-D") into p57, thereby yielding plasmid p58. *See id.* In p58, the gene was also positioned adjacent to the spacer sequence, such that the leader, spacer, and gene sequences were all oriented in a fashion identical to the claimed construct. From September 6 to 11, 1982, Singh's assistant, Dr. June Lugovoy, isolated the DNA segment from p58 containing the alpha-factor leader, spacer, and IFN-D sequence, and inserted that segment (hereinafter "the p60 DNA construct") into yeast plasmid YEp9PT ("p60"). *See id.* ¶ 26. Plasmid p60 was then introduced into yeast cells to determine whether the p60 DNA construct would generate IFN-D. *See id.* ¶ 27.

On October 1, 1982, protein sequencing chemist Bill Kohr informed Singh that the IFN-D expressed by yeast cells transformed with p60 contained eight additional amino acids not normally present in natural IFN-D. *See id.* ¶ 33. On approximately that same date, Singh alleges that he conceived the claimed DNA construct, *i.e.*, he devised a plan to redesign the p60 DNA construct in order to obtain the desired gene product, IFN-D, free of those additional amino acids. *See id.* ¶ 34. Specifically, Singh claims that he realized that he would need to remove eight unwanted codons (twenty-four nucleotides) from the p60 DNA construct, and that he planned to accomplish this deletion by use of a technique known as "loop deletion mutagenesis."

On November 24, 1982, Singh wrote a laboratory notebook entry setting forth the undesired eight codons in the p60 DNA construct, as well as the twelve nucleotides on either side of that eight codon segment (the "flanking sequences"). *See* Singh Decl. ¶ 45. On that date, Singh also ordered a linear, 24-nucleotide sequence (a "24-mer") that comprised the nucleotides of the flanking sequences.[3] This order was can-

factor and signals that the peptide is to be exported from the cell. *See* U.S. Application 06/506,098 at 3, ll. 3-5. That sequence is typically removed from alpha-factor upon secretion. *See id.* at 3, ll. 1-3. It is the alpha-factor leader sequence alone that is incorporated into the claimed construct.

[2] A "codon" is a set of three nucleotides that codes for a particular amino acid.

[3] Actually, this statement is incorrect. The 24-mer sequence that Singh ordered on November 24, 1982, was not identical to the nucleotides of the flanking sequences, but instead included several "preferred codons."

celed on the same day, and a notation in Singh's laboratory notebook stated that Singh would perform the deletion experiment in a different way "without changing codons." *Id.* On December 1, 1982, Singh ordered another 24-mer for the deletion experiment. This 24-mer was precisely complementary to the flanking sequences set forth in the November 24 entry. *See* Singh Decl. ¶ 47. DNA chemist Peter Ng testified that he synthesized the 24-mer for Singh on December 20, 1982. *See* Ng Decl. ¶ 11; Ng Dep. at 36. Singh affixed the order into his notebook on December 21, 1982, with a notation "oligonucleotide for making in-frame deletion of alpha pro-IFN-D junction."[4] Singh alleges that these facts corroborate his testimony that he conceived the claimed DNA construct before January 12, 1983, the filing date of Brake 1.

Id. at 1364-65, 55 USPQ2d at 1674-75 (footnote omitted).

At the final hearing on May 11, 1998, Singh sought: (1) to contest the interlocutory order granting Brake the benefit of Brake 1; (2) to prove Singh's conception of the invention of the count prior to Brake 1's January 12, 1983 filing date; and (3) to show diligence throughout the "critical period" from just prior to January 12, 1983, until actual reduction to practice. Singh was unsuccessful with respect to all three issues, and final judgment was issued in favor of Brake on August 31, 1998. *Brake*, Paper No. 164.

Singh appealed to this court, contesting Brake's entitlement to the benefit of Brake 1 and contesting the Board's finding that Singh had failed to prove conception prior to the Brake 1 filing date. We held that certain of the Board's key findings underlying its conclusion that Singh had failed to prove conception of the subject matter of the interference prior to the effective filing date of Brake were unsupported by substantial evidence, and we vacated and remanded. *Singh*, 222 F.3d at 1370, 55 USPQ2d at 1679. We also found that the Board did not address whether Brake 1 adequately described and enabled the disputed subject matter of the count under 35 U.S.C. § 112, ¶ 1, and we remanded for determination of those issues as well. *Id.* at 1371, 55 USPQ2d at 1679.

On remand, the Board permitted the parties to submit briefs on the remanded issues, but returned Singh's enablement and written description briefs (as well as Brake's corresponding reply briefs) with its opinion, stating that Singh had failed to comply with the requirements of 37 C.F.R. § 1.655(a) and (b) by presenting new arguments not raised in the original opposition.

In an eighty-nine-page opinion with an additional seventeen-page concurrence, *Brake*, Paper No. 199, the Board addressed each of the issues on remand and concluded: (1) that Brake 1 adequately described and enabled the invention of the count, and Brake was therefore entitled to the benefit of Brake 1's filing date; (2) that Singh had not met his burden of proving conception prior to the filing date of Brake 1; and (3) that even if it were assumed, *arguendo*, that Singh had conceived the invention prior to Brake's filing date, Singh had not met his burden of demonstrating diligence between conception and reduction to practice.

Singh now appeals again. We have jurisdiction pursuant to 35 U.S.C. § 141 and 28 U.S.C. § 1295(a)(4)(A) (2000).

DISCUSSION

A. Return of Briefs

Pursuant to our earlier decision's remand "for determination of those issues that were properly raised during the earlier proceedings," *Singh*, 222 F.3d at 1371, 55 USPQ2d at 1679, the Board invited the parties to submit briefs on the issues of Singh's case for priority and Brake's sustenance of his burden of proof with respect to written description and enablement. *Brake*, Paper No. 199 at 12. After the parties submitted the invited briefs, the Board determined that Singh had presented new arguments in derogation of the Board's reminder that only issues that were properly raised in the original opposition were entitled to review at the final hearing. *Id.* at 13. In response, the Board returned all of the newly submitted briefs to the parties without further consideration, holding that the briefs contained, "almost exclusively, new arguments, and lack the showing that Preliminary Motion 2 [concerning the Brake patent's entitlement

[4] This point is disputed. Singh has provided no corroboration of his assertion that this notation was actually made on December 21, 1982. Like the other pages of Singh's notebook, this page was not witnessed until 1986, and, even then, there is no proof that the notation existed at the time of the witnessing.

to the Brake 1 filing date] should be modified." *Id.* at 15.

[1] Singh argues that the Board erred in refusing to consider briefs submitted by Singh on remand. We review the Board's application of its rules for an abuse of discretion. *Brown v. Barbacid*, 276 F.3d 1327, 1332, 61 USPQ2d 1236, 1238 (Fed. Cir. 2002). Although returning the briefs to the parties is a rather extraordinary measure, we do not find any abuse of discretion in the Board's doing so. 37 C.F.R. § 1.655(b) states:

> A party shall not be entitled to raise for consideration at final hearing any matter which properly could have been raised by a motion under § 1.633 or 1.634 unless the matter was properly raised in a motion that was timely filed by the party under § 1.633 or 1.634 and the motion was denied or deferred to final hearing, the matter was properly raised by the party in a timely filed opposition to a motion under § 1.633 or 1.634 and the motion was granted over the opposition or deferred to final hearing, or the party shows good cause why the issue was not properly raised by a timely filed motion or oppositions.

37 C.F.R. § 1.655(b) (2002).

Because the Board found that Singh was attempting to raise in his briefs matters that could have been but were not raised at the outset of the interference, *see Brake*, Paper No. 199 at 12, the Board was acting properly within its discretion when it refused to consider the briefs. Singh could have raised his written description and enablement arguments at the outset of the interference; to the extent that he did not do so, those arguments have been waived. As we stated in *Credle v. Bond*, 25 F.3d 1566, 30 USPQ2d 1911 (Fed. Cir. 1994), the Board does not abuse its discretion when it declines to consider untimely arguments. *Id.* at 1572 n.14, 30 USPQ2d at 1916 n.14. Furthermore, because the Board explicitly stated in its November 2, 2000 order that additional briefing was optional, *Brake*, Paper No. 179 at 5, it is difficult to see how the subsequent refusal to consider the briefs could have been an abuse of discretion.

Singh also asserts that the Board refused to consider certain arguments made in his "original" Main Brief. We find no abuse of discretion. Again, Singh did not show good cause for failing to raise these arguments at the preliminary motion stage, and the Board was entitled to decline to consider them.

B. Conception and Reduction to Practice

"Conception is the formation 'in the mind of the inventor of a definite and permanent idea of the complete and operative invention, as it is therefore to be applied in practice.' " *Kridl v. McCormick*, 105 F.3d 1446, 1449, 41 USPQ2d 1686, 1689 (Fed. Cir. 1997) (citations omitted). A conception must encompass all limitations of the claimed invention, *see id.*, and "is complete only when the idea is so clearly defined in the inventor's mind that only ordinary skill would be necessary to reduce the invention to practice, without extensive research or experimentation," *Burroughs Wellcome Co. v. Barr Labs. Inc.*, 40 F.3d 1223, 1228, 32 USPQ2d 1915, 1919 (Fed. Cir. 1994).

Priority of invention and its constituent issues of conception and reduction to practice are questions of law predicated on subsidiary factual findings. *Brown*, 276 F.3d at 1332, 61 USPQ2d at 1238; *Hitzeman v. Rutter*, 243 F.3d 1345, 1353, 58 USPQ2d 1161, 1166 (Fed. Cir. 2001). Accordingly, we review *de novo* the Board's legal conclusions with respect to priority, conception, and reduction to practice, 5 U.S.C. § 706 (2000); *Brown*, 276 F.3d at 1332, 61 USPQ2d at 1238; *Hitzeman*, 243 F.3d at 1353-54, 58 USPQ2d at 1166-67, and we review factual findings by the Board for substantial evidence, *Dickinson v. Zurko*, 527 U.S. 150 [50 USPQ2d 1930] (1999); *In re Gartside*, 203 F.3d 1305, 1315, 53 USPQ2d 1769, 1775 (Fed. Cir. 2000).

A junior party whose effective filing date is earlier than the date the senior party's patent issued and who is seeking a determination of priority must demonstrate by a preponderance of the evidence either reduction to practice before the senior party's priority date, or prior conception coupled with reasonable diligence in reducing the invention to practice from a time just prior to the senior party's entry into the field to the junior party's own reduction to practice. 35 U.S.C. § 102(g) (2000); *Griffin v. Bertina*, 285 F.3d 1029, 1032, 62 USPQ2d 1431, 1433 (Fed. Cir. 2002); *Mahurkar v. C.R. Bard, Inc.*, 79 F.3d 1572, 1577, 38 USPQ2d 1288, 1290 (Fed. Cir. 1996).

It is well established that when a party seeks to prove conception via the oral testimony of a putative inventor, the party must

proffer evidence corroborating that testimony. *See Mahurkar*, 79 F.3d at 1577, 38 USPQ2d at 1290; *Price v. Symsek*, 988 F.2d 1187, 1194, 26 USPQ2d 1031, 1036 (Fed. Cir. 1993). That rule addresses the concern that a party claiming inventorship might be tempted to describe his actions in an unjustifiably self-serving manner in order to obtain a patent or to maintain an existing patent. *See Eibel Process Co. v. Minn. & Ont. Paper Co.*, 261 U.S. 45, 60 (1923); *Kridl*, 105 F.3d at 1450, 41 USPQ2d at 1689 ("The tribunal must also bear in mind the purpose of corroboration, which is to prevent fraud, by providing independent confirmation of the inventor's testimony."); *Price*, 988 F.2d at 1194-95, 26 USPQ2d at 1036-37. There is no particular formula that an inventor must follow in providing corroboration of his testimony of conception. *See Kridl*, 105 F.3d at 1450, 41 USPQ2d at 1689. Rather, whether a putative inventor's testimony has been sufficiently corroborated is determined by a "rule of reason" analysis, in which "an evaluation of all pertinent evidence must be made so that a sound determination of the credibility of the inventor's story may be reached." *Price*, 988 F.2d at 1195, 26 USPQ2d 1031 at 1037. However, that "rule of reason" analysis does not alter the requirement of corroboration of an inventor's testimony. *Brown*, 276 F.3d at 1335. Evidence of the inventive facts must not rest alone on the testimony of the inventor himself. *Cooper v. Goldfarb*, 154 F.3d 1321, 1330, 47 USPQ2d 1896, 1903 (Fed. Cir. 1998).

Singh argues that the Board did not consider the totality of the corroborative evidence establishing Singh's conception, but only considered individual pieces of evidence in "total isolation from one another." Specifically, Singh argues that his November 24, 1982 notebook entry and his ordering of the specific 24-mer oligonucleotide ultimately used to carry out the loop deletion mutagenesis method (in February 1983) establish that he had a definite and permanent idea of the structure of a DNA construct within the count and of an operative way of making it prior to Brake 1's filing date.

We disagree. First, as we stated in our earlier opinion, *Singh*, 222 F.3d at 1368, 55 USPQ2d at 1677, the Board correctly held as a matter of law that Singh failed to prove that he conceived the claimed construct prior to December 1, 1982. In his November 24, 1982 notebook entry, Singh identified the twenty-four nucleotides encoding the eight extraneous amino acids present in the IFN-D generated by the p60 DNA construct, labeling them with the notation, "sequence to be removed." He also identified in that entry the twelve nucleotides immediately upstream and the twelve nucleotides immediately downstream from those twenty-four, *i.e.*, the flanking segments. Accordingly, he may have articulated in that entry the problem to be solved, namely, the need to eliminate the twenty-four nucleotides encoding the extraneous amino acids. Nonetheless, substantial evidence supports the Board's finding that that entry alone was insufficient to corroborate Singh's testimony. Even if the entry expressed the problem, it did not provide the solution. *See Brake*, Paper No. 164 at 22-24. The Board's key findings in this regard, both of which are supported by substantial evidence in the notebook entry itself, are: (1) that a linear 24-mer other than the one necessary to accomplish the deletion was first ordered, and (2) that the order was canceled the same day, with a notation "will do in a different way and w/o changing codons." *Id.* at 23-24.

Secondly, as noted above, the 24-mer sequence that Singh initially ordered on November 24, 1982, was not identical to the nucleotides of the flanking sequences. Instead, he included several "preferred codons," casting doubt on the accuracy of Singh's statement that he ordered that 24-mer "[i]n order to remove this sequence by oligonucleotide deletion mutagenesis." While it remains unclear exactly what Singh "planned" to do on November 24, 1982, his identification of preferred codons suggests to us that his plans may not have included the use of loop deletion mutagenesis.

The Board duly considered the fact that the 24-mer ordered by Singh on December 1, 1982, was indeed complementary to the four codons on each side of the sequence Singh allegedly desired to delete. *See, e.g., Brake*, Paper No. 199 at 13-14, 19, 58-59, 77-78. The Board also reviewed Singh's notebook pages purporting to demonstrate conception. The Board concluded, and we agree, that "Singh's entire case for conception rests on the order of a 24-mer and an uncorroborated notation in a corner of Dr. Singh's notebook." *Id.* at 84.

[2] There is nothing in Singh's notebook that corroborates his testimony that the No-

vember 24, December 1, and December 21 entries were meant to be read together. Even viewing all of these entries together, however, we find that the sum falls short of proving by a preponderance of the evidence that Singh had a definite and permanent idea of an operative method of making the DNA construct of the count prior to Brake 1's filing date. As the Board observed, the notebook entries do not provide any protocol or outline of the loop deletion mutagenesis procedure: "At best, the notation states a goal which Dr. Singh hopes to achieve; i.e., an in-frame deletion of the á pro-IFN-D junction." *Id.* at 61. Adelman et al., *In Vitro Deletional Mutagenesis for Bacterial Production of the 20,000-Dalton Form of Human Pituitary Growth Hormone*, 2 DNA 183 (1983), which described the loop deletion mutagenesis procedure, also described using oligonucleotides complementary to nucleotide sequences flanking codons to be deleted *as probes* for identifying plasmids from which the codons had been deleted. *Id.* at 188. We find it no less plausible that Singh was ordering the 24-mer for use as a probe than it was that he was ordering it for use in the loop deletion mutagenesis procedure. Indeed, Singh has pointed to no evidence in the record in support of his assertion that loop deletion mutagenesis was developed at Genentech in late 1982 (the Adelman et al. paper was published in 1983), let alone that Dr. Singh knew of any such developments prior to Brake 1's filing date. The burden was on Singh to prove that he as the inventor had a definite and permanent idea of how to make the construct. *See Coleman v. Dines*, 754 F.2d 353, 360, 224 USPQ 857, 863 (Fed. Cir. 1985). That he did not do.

Finally, we address Singh's argument set forth in his brief that, "[w]ith respect to the issue of conception, this Court previously made specific findings ... that Singh articulated a specific plan to design the claimed construct by the loop deletion method on November 24, 1982." That statement is a mischaracterization of our earlier opinion, in which we simply said that the Board needed to consider the totality of the evidence, including evidence of Singh's identification of the "sequence to be removed" and the twelve nucleotides immediately upstream and downstream from this sequence, as well as of his ordering of a 24-mer identical to the sequences flanking the undesired sequence. We are satisfied that the Board has done so.

Thus, after review of the record evidence in light of the proper legal standards, we conclude that substantial evidence supports the Board's key finding that no evidence links the nucleotide Singh ordered on December 1, 1982, with a plan to design the claimed construct prior to January 12, 1983.

Because we find that Singh did not meet his burden of demonstrating conception prior to Brake 1's filing date by a preponderance of the evidence, we need not address Singh's arguments regarding reduction to practice. However, we note the Board's finding that, apart from attorney argument, "Singh's evidence of diligence primarily consists of various pages from Dr. Singh's laboratory notebook which are (i) unexplained as to content and relevance to the invention of the Count, and (ii) uncorroborated." *Brake*, Paper No. 199 at 88. We agree that Singh's activities completed on December 20, 1982, were the only relevant, corroborated activities performed by Singh prior to Brake 1's January 12, 1983, filing date, and, as a result, Singh failed to prove reasonable diligence toward reduction to practice by a preponderance of the evidence.

C. Written Description and Enablement

Whether a specification supports a claim corresponding to a count, and thus satisfies the written description requirement of 35 U.S.C. § 112, ¶ 1, is a question of fact, *Vas-Cath v. Mahurkar*, 935 F.2d 1555, 1562, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991), and is, in appeals from the United States Patent and Trademark Office, reviewed under the substantial evidence standard. *In re Gartside*, 203 F.3d at 1315, 53 USPQ2d at 1775. Singh argues that the Board erred in concluding that Brake is entitled to the benefit of the Brake 1 application. First, Singh contends that Brake did not provide an adequate written description of the invention of the count in the Brake 1 application, and should not be entitled to its benefit. According to Singh, Brake 1 disclosed a large genus, allegedly encompassing over 9000 species[5] (n is 0 or 1 to 4), of which the count is directed to only two (*i.e.*, those where n = 0).

[5] Singh bases that number on the formula "$((R)_r$-$(GAXYCX)_n$-Gene*$)_y$" disclosed at page 3, line 33, of Brake 1, in which R = CGX or AZZ; r = "an integer of from 2 to 4, . . ., preferably 2"; X = T, G, C, or A; Y =

Secondly, Singh asserts that Brake 1 does not provide an enabling disclosure with respect to the invention of the count, arguing that Brake 1 does not disclose how to make and use the "n = 0" embodiment, and that "the techniques which were available to Brake at the time of filing the Brake 1 Application were not sufficient to obtain the DNA constructs of the Count." Singh also argues that Brake 1 "is replete with passages which guide one of ordinary skill in the art to constructs wherein n > 0, which constructs are not encompassed by the Count." Finally, Singh argues that "during prosecution of the Brake 2 Application, Brake argued that the results obtained with the n = 0 construct were unexpected, because those of ordinary skill in the art believed that the Glu-Ala sequences were required."

[3] Singh's arguments are not persuasive. First, we disagree with Singh's argument that the invention of the count represents just two of 9000+ species disclosed in Brake 1. Singh's calculation of 341 permutations for $(GAXYCX)_n$ is apparently based on an unwarranted assumption that each iteration of the parenthetical sequence is independently chosen. However, as Brake pointed out, because the variable 'n' is outside the parentheses, $(GAXYCX)_n$ can code for either no amino acids (*i.e.*, when n = 0), or 1 to 4 copies of one of four different amino acid sequences (*i.e.*, Asp-Pro, Asp-Ala, Glu-Pro, or Glu-Ala). *Brake*, Paper No. 199 at 20-21 n.13. Thus, there are at most only seventeen (*i.e.*, $4^0 + 4^1 + 4^1 + 4^1 + 4^1$) permutations of that sequence. Even among those seventeen, however, we agree with Brake that there are only two meaningful embodiments: one in which a dipeptidylaminopeptidase A (DPAP) signal is present (*i.e.*, n = 1 to 4), and one in which it is not (*i.e.*, n = 0).

Moreover, Singh's calculation of twenty-eight possibilities for the Lys/Arg sequences is artificially inflated because it ignores the disclosure of claim 5 of Brake 1:

> 5. A DNA construct comprising a sequence of the following formula:
>
> $L\text{-}(R\text{-}S\text{-}(GAXYCX)_n\text{-}Gene^*)_y$
>
> wherein:
>
> L is a leader sequence recognized by yeast for secretion;
>
> R and S are codons coding for arginine and lysine;
>
> X is any nucleotide;
>
> Y is guanosine or cytosine;
>
> y is an integer of from about 1 to 10;
>
> Gene* is a gene foreign to yeast; and
>
> n is 0 or 1 to 4.

U.S. Application 06/457,325 at 16, ll. 20-32.

In claim 5, spacer R-S encodes four possible sequences (*i.e.*, Lys-Arg, Arg-Arg, Arg-Lys, or Lys-Lys), not 28. Of these four, two permutations, Lys-Arg and Arg-Arg, are within the scope of the count.

Singh cites *Fujikawa v. Wattanasin*, 93 F.3d 1559, 39 USPQ2d 1895 (Fed. Cir. 1996), for the proposition that an application disclosing a generic chemical formula must provide adequate direction to those of ordinary skill in the art to lead them to a subgenus of the proposed count. We find Singh's reliance on *Fujikawa* to be unsound. In *Fujikawa*, we held that disclosure of a generic quinoline structure with four variable groups, each of which could be independently chosen from a list of functional groups, provided insufficient written description support for a count directed to a subgeneric structure having a single combination of the four groups. *Id.* at 1569-71, 39 USPQ2d at 1904-05. However, Brake 1's formula does not present the same issue as did the quinoline in *Fujikawa*. First, replacing a functional group on a chemical compound can often have highly unpredictable results. We noted in *Fujikawa* that even a change as seemingly trivial as replacing an isopropyl group with the isosteric cyclopropyl group at issue in that case could result in either a significant improvement or reduction in the activity of the compound against a particular biological target. *Id.* In the present case, on the other hand, as mentioned above, there are only two subgenera that are biologically relevant: one in which a DPAP signal is present (*i.e.*, n = 1

G or C; y = "an integer of least one and usually not more than 10, more usually not more than four . . ."; Z = A or G; and n = "0 or an integer which will generally vary from 1 to 4, usually 2 to 3."

According to Singh, Each "R" can encode either Lys or Arg, so (R)r can encode twenty-eight (*i.e.*, $2^2 + 2^3 + 2^4$) different amino acid sequences. In addition, each "GAXYCX" sequence can encode any of four amino acid sequences: Asp-Pro, Asp-Ala, Glu-Pro, or Glu-Ala, so $(GAXYCX)_n$ can encode 341 (*i.e.*, $4^0 + 4^1 + 4^2 + 4^3 + 4^4$) different amino acid sequences. Thus, Singh argues that the Brake 1 formula covers 9548 (*i.e.*, 28 x 341) different species.

to 4), and one in which it is not (*i.e.*, n = 0), a simpler case than in *Fujikawa*. Here, moreover, claim 5 of Brake 1 discloses that "n is 0 or 1 to 4," which is a clear "blaze mark" providing *in ipsis verbis* support for "n = 0" in the count. *In re Ruschig*, 379 F.2d 990, 994-95, 154 USPQ 118, 122 (CCPA 1967).

The Supreme Court has explained that "the possibility of drawing two inconsistent conclusions from the evidence does not prevent an administrative agency's finding from being supported by substantial evidence." *In re Gartside*, 203 F.3d at 1312, 53 USPQ2d at 1773 (citing *Consolo v. Federal Maritime Comm'n*, 383 U.S. 607, 620 (1966)). In *Fujikawa*, we said that "[w]hile Fujikawa's arguments are not without merit, we cannot say, on this record, that the Board's decision was clearly erroneous." 93 F.3d at 1571, 39 USPQ2d at 1905. In view of the fact that the "substantial evidence" standard of review that we now use post-*Zurko* requires us to give decisions of the Board greater deference than we gave in cases such as *Fujikawa*, we likewise decline to find legal error in the Board's conclusion on the record in the present case.

Singh's reliance on *Bigham v. Godtfredsen*, 857 F.2d 1415, 8 USPQ2d 1266 (Fed. Cir. 1988), is also unavailing. In *Bigham*, Godtfredsen's first application disclosed a compound having a substituent "X", where X was defined as "a halogen atom." The application provided as its only example a compound in which X was chloro. *Id.* at 1416, 8 USPQ2d at 1267. This court ruled that that application's disclosure of "halogen" did not meet the requirements of § 112 as a written description of bromo or iodo species, particularly where Godtfredsen had earlier argued in the same case that bromo and iodo were patentably distinct from chloro in order to urge bifurcation of the count. *Id.* at 1417, 8 USPQ2d at 1268. In the present case, in contrast, "n = 0" was disclosed in Brake 1. If Godtfredsen had provided examples of fluoro, bromo, and iodo compounds in addition to the chloro compound, that case might have been decided differently, even in spite of Godtfredsen's "patentably distinct" argument.

Singh's arguments with respect to enablement are likewise unconvincing. Enablement is a question of law based on underlying factual determinations. *In re Swartz*, 232 F.3d 862, 863, 56 USPQ2d 1703, 1704 (Fed. Cir. 2000). We review the Board's underlying findings of fact for substantial evidence, and review *de novo* its ultimate conclusion whether a disclosure is enabling. *Id.* Singh argues in his brief:

> The Board takes internally inconsistent positions with respect to whether methods for obtaining a construct of the Count using Brake's starting material were available to those of ordinary skill in the art at the time the Brake 1 Application was filed. To support its finding that Brake is entitled to benefit, the Board finds that such methods existed. However, to support its finding that Singh had not conceived of the invention prior to the Brake 1 Application filing date, the Board makes the contrary finding.

[4] We find no error or inconsistency in the Board's analysis. As we wrote in *Glaxo Inc. v. Novopharm Ltd.*, 52 F.3d 1043, 1050, 34 USPQ2d 1565, 1569 (Fed. Cir. 1995), "the enablement requirement . . . looks to the objective knowledge of one of ordinary skill in the art." *Id.* (citing *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1532, 3 USPQ2d 1737, 1742 (Fed. Cir. 1987)). Thus, whereas the test for determining whether or not Singh conceived the construct of the count depended on Singh's own personal knowledge of methods for making the construct and his formulation of a definite and permanent idea therefor, whether Brake 1 enables an invention within the count does not depend on what Brake knew, but rather on whether the application enables one skilled in the art to make and use the invention, *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), "at the time the patent application was filed." *Ajinomoto Co. v. Archer-Daniels-Midland Co.*, 228 F.3d 1338, 1345, 56 USPQ2d 1332, 1337 (Fed. Cir. 2000). The Board found that the testimony of Brake's witness, Dr. Patricia Tekamp-Olson, demonstrated that those of ordinary skill in the art had in their possession in 1982 various molecular biological methods sufficient to make and use the "n = 0" construct, including site-directed mutagenesis. *Brake*, Paper No. 199 at 24-27. The Board also found that Singh's expert, Dr. Joseph Falkinham, mischaracterized the teachings of the Fritz article on which he relied in his attempts to discredit Tekamp-Olson's testimony. *Id.* at 40.

As further "proof" that Brake 1 does not provide an enabling disclosure of the inven-

tion of the Count, Singh also alleges, for example, that "the Brake 1 Application actually steers the artisan to species clearly outside the Count," that "during prosecution of the Brake 2 Application, Brake argued that the results obtained with the n = 0 construct were unexpected," and that "Dr. Brake did not realize the disadvantages of the n > 0 constructs until well after the Brake 1 Application was filed." We are not persuaded by any of these arguments, and conclude that Singh has apparently confused the criteria for proving obviousness with those for demonstrating that a disclosure is nonenabling. Although the questions (1) whether or not a reference "teaches away" from a claimed invention and (2) whether or not a claimed invention provides "unexpected results" are relevant in determining whether or not a claimed invention would have been obvious, *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1550, 220 USPQ 303, 311 (Fed. Cir. 1983), they are not the primary questions bearing on enablement. The fact that the Brake patent states that n in the construct is "preferably 2 or 3" is also irrelevant to the question of enablement of the n = 0 construct.[6] Similarly, the fact that the n = 0 construct might have had after-discovered advantages over the n > 0 constructs has no bearing at all on whether or not Brake 1 contained an enabling disclosure.

We thus conclude that substantial evidence supports the Board's finding that Brake was entitled to the benefit of the Brake 1 application. We have considered Singh's other arguments and do not find them persuasive.

CONCLUSION

Because the Board's decision was supported by substantial evidence and contained no errors of law, the Board did not err in concluding that Singh failed to show (1) that Brake was not entitled to the Brake 1 filing date and (2) that Singh reduced the invention to practice before Brake's priority date. The Board's decision to award judgment to Brake is therefore

AFFIRMED.

[6] The Board properly discredited Falkinham's testimony on that point. Paragraph 9 of Falkinham's Declaration states: "Although there was a theoretical presentation of the n=0 construct in the Brake 1 application, there was a clear statement that 'n' in the construct was 'preferably 2 or 3' (column 3, line 25) or 'usually 2 or 3' (column 2, line 68) . . . One skilled in the art would have determined from the Brake specification that the n=0 construct was not desirable." As the Board noted, *Brake*, Paper No. 199 at 36, Falkinham's citations to "columns" 2 and 3 obviously refer to the Brake patent (of which claim 1 is identical to the Count in this interference), and not to Brake 1.

Time Warner Entertainment Co. v. Jones

U.S. Patent and Trademark Office
Trademark Trial and Appeal Board

Opposition No. 112,409

Decided July 17, 2002

TRADEMARKS AND UNFAIR TRADE PRACTICES

[1] Practice and procedure in Patent and Trademark Office — Interpartes proceedings — Opposition and cancellation — Rules and rules practice (§ 325.0305.05)

JUDICIAL PRACTICE AND PROCEDURE

Procedure — Evidence — In general (§ 410.3701)

Procedure — Discovery — Interrogatories (§ 410.4005)

Trademark applicant's objection to opposer's deposition testimony, on ground that opposer refused to "identify each and every fact, document and witness in support of its pleaded allegations" as requested in applicant's interrogatories, is overruled, since applicant's requests are equivalent to improper request for identification of fact witnesses and trial evidence prior to trial, since applicant has not been deprived of opportunity for discovery, or subjected to "trial by ambush," and since applicant did not formally or clearly raise objection until she filed her brief, and therefore has waived her objection to opposer's testimony deposition evidence and attached exhibits.

Patent
Attorney's Docket No. 010057-005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Singh	)	Interference No.: 102,728
v.	)	Administrative Patent Judge: Ronald H. Smith
Brake	)	

DECLARATION OF DR. ARJUN SINGH
PURSUANT TO 37 C.F.R. § 1.672(b)

Box: Interference
Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Arjun Singh, hereby declare as follows:

1. I am the sole inventor of the subject matter claimed in U.S. Patent Application Serial No. 06/506,098 filed June 20, 1983 and its continuation, U.S. Serial No. 07/552,719, filed July 16, 1990.

2. I am a citizen of the United States residing at 735 Cape Breton Drive, Pacifica, California 94044.

3. I was employed as a research scientist and Scientific Manager by Genentech, Inc. ("Genentech") located at 460 Point San Bruno Boulevard, South San Francisco, California 94080 from 1981 to 1991.

4. From 1992 to the present, I have been employed by Genotypes, Inc., located at 61 Airport Boulevard, Suite B, South San Francisco, California 94080 as the Director of Molecular Genetics.

5. My qualifications, which are more fully set out in my *curriculum vitae* (Singh Exhibit 1, Bates Nos. 000509-513), include a Ph.D. in genetics awarded in 1969 by the University of Illinois at Champaign-Urbana and over 24 years of experience in genetics and molecular biology of yeast.

6. It was my practice in 1982 and 1983 to write the date that an experiment was carried out at the bottom of the page of my Notebook. When I entered data that were generated by others into my Notebook, I generally entered the date that such data were put into my Notebook at the bottom of the page.

7. As part of my job in 1982 and 1983, I supervised June Lugovoy's work very closely and directed her research.

genes code for α-factor peptides. The α-factor peptide derived from the major gene is initially produced by the yeast cell as a prepro poly α-peptide consisting of 165 amino acids. During processing in the yeast cell, a region at the N-terminus of the peptide consisting of 19 or 20 amino acids is cleaved from the peptide, resulting in a peptide of 145 amino acids. The sequence removed is commonly referred to as the "pre" region or signal peptide. The signal peptide is followed by a "pro" segment of approximately 60 amino acids. The second half of the α-factor precursor contains four tandem copies of mature α-factor precursor, each preceded by a spacer peptide. Processing of the α-factor precursor by removal of the spacer peptides yields four mature α-factors (Singh Exhibit 28, Bates Nos. 000514-000528; Singh Exhibit 5, Bates Nos. 000491-000501).

12. By June 1, 1982 I had isolated two DNA fragments containing α-factor genes from *S. cerevisiae*: a 1.7 kb EcoRI fragment and a 1.8 kb HindIII fragment (Singh Exhibit 2, Notebook 1007, Bates Nos. 000001-000008, 000011-000015, 000017). On June 1-2, 1982, I recorded that I had ligated the isolated fragments to appropriately cleaved plasmid pBR322 DNA. I used the ligation mixture to transform *E.coli* 294 (Singh Exhibit 2, Notebook 1007, Bates Nos. 000018-000019).

13. I screened 40 transformed *E.coli* cultures to identify the ones which contained the α-factor gene. (Singh Exhibit 2, Notebook 1007, Bates No. 000020.)

On June 5, 1982 I explain on Bates page 000021 of my Notebook (page 57) that the HindIII fragments from DNA of clone #14, identified on Bates page 000017 of my Notebook (page 53), had been successfully cloned but that cloning of EcoRI fragments from the DNA of clone #16 was not successful. I therefore reisolated EcoRI fragments from clone #16 and repeated the ligation into pBR322 as previously described. (Singh Exhibit 2, Notebook 1007, Bates Nos. 000022-000024). Bates page 000025 of my Notebook shows that by June 21, 1990 I had transformed colonies with the new EcoRI fragments (page 71). On Bates page 000026 (Singh Exhibit 2, Notebook 1007), a gel is shown which demonstrates that the EcoRI fragments were successfully cloned in 4 independent colonies.

14. On June 23, 1982 I verified which clones had correct DNA inserts by hybridization with the labeled oligonucleotide probes. (Singh Exhibit 2, Notebook 1007, Bates Nos. 000027-000029.)

15. As recorded on June 27, 1982, at the bottom of Bates page 000029 (Singh Exhibit 2, Notebook 1007) the plasmid containing the HindIII fragment in pBR322 was named p54, while the plasmid containing the EcoRI fragment was named p53. June Lugovoy's Notebook shows the restriction map of p53 (Singh Exhibit 20, Notebook 861, Bates No. 000415).

16. From June 28 to June 29, 1982 I digested the DNA from p53 and p54 with various enzymes to construct their restriction enzyme maps (Singh Exhibit 2, Notebook 1007, Bates Nos. 000030-000031). The restriction enzyme maps I made for p53 and p54 were recorded by me on July 1, 1982 on page 81 of Notebook 1007 (Singh Exhibit 2, Bates no. 000032.) I could use these maps to design the next plasmid constructs containing the α-factor genes and to obtain DNA fragments of the α-factor genes for sequencing.

17. On July 1, 1982 I recorded on the bottom of Bates page 000032 (Singh Exhibit 2, Notebook 1007) the next steps that had to be done: 1) run acrylamide gel for smaller fragments; 2) perform double digests that cut in inserts; and 3) find out what additional information was needed for sequencing the α-factor gene.

18. From June 29, 1982 to July 5, 1982, I retested p53 and p54 for hybridization with the probes and found on July 5, 1982, that p53 hybridized but p54 did not hybridize as shown on Bates pages 000033-000034 (Singh Exhibit 2, Notebook 1007). Because I had not yet cloned the 1.8 kb HindIII fragment, I began preparing p54 over again on July 10, 1982. (Singh Exhibit 2, Notebook 1007, Bates No. 000035). The resulting plasmid DNA was available by July 17, 1982 and was named p56. (Singh Exhibit 2, Notebook 1007, Bates No. 000036-000038.) I then mapped p56 for several restriction enzyme sites and confirmed the presence of the hybridizing

sequence by a "dot blot" procedure from July 24 to July 29, 1982. (Singh Exhibit 2, Notebook 1007, Bates Nos. 000039-000045, Singh Exhibit 3, Notebook 1249, Bates No. 000050.)

19. I confirmed that new plasmid p56 contained the 1.8 kb HindIII fragment insert of yeast α-factor. Plasmid p53 contained the 1.7 kb EcoRI fragment insert of yeast α-factor. These fragments were later referred to by me respectively as MFα2 and MFα1 as shown in Singh Exhibit 3, which represents selected pages from my Notebook No. 1249. (Bates Nos. 000046-000055, 000058-000059.)

20. I hypothesized that the plasmids containing MFα1 promoter and prepro/spacer sequences could be used for expression and secretion of human proteins in yeast cells.

21. On August 6, 1982, I devised a method of creating a general expression vector for expressing and secreting human polypeptides using the α-factor promoter and the prepro and spacer sequences from p53 (MFα1). (Singh Exhibit 3, Notebook 1249, Bates Nos. 000060-000062.) On my instructions, June Lugovoy removed the DNA sequences coding for the α-factor structural gene from p53 so that the resulting plasmid, p57, contained the promoter sequences and the sequence corresponding to 89 amino acids of the α-factor "prepro/spacer" protein. (Singh Exhibit 3, Notebook 1249,

Bates Nos. 000064-000065.) The DNA fragment containing the prepro/spacer α-factor sequence was to be ligated to a DNA fragment containing the human interferon D (IFN-D) sequence and the plasmid pBR322 in a three-way ligation to form p58 (Singh Exhibit 3, Notebook 1249, Bates Nos. 000060-000061). From August 12 to August 21, 1982, I carried out this plan (Singh Exhibit 3, Notebook 1249, Bates Nos. 000066-000068). The Notebook of J. Lugovoy shows the restriction map of p58. (Singh Exhibit 20, Notebook 861, Bates No. 000417)

22. On August 21, 1982, I transformed *E.coli* 294 with the ligation mixture for p58 and directed June Lugovoy to screen 40 colonies of transformants for correct insertion and orientation. (Singh Exhibit 3, Notebook 1249, Bates Nos. 000069-70.)

23. The property of growth inhibition of "a" yeast cells by α-factor was used to test whether or not the pheromone gene contained in the cloned 1.7 kb EcoRI and 1.8 kbp HindIII fragments was functional. If an active α-factor pheromone gene were present in a plasmid, it would be expected that significantly more pheromone would be synthesized in yeast cells containing a multi-copy plasmid than in yeast cells containing only the chromosomal copy (or copies) of the gene. The enhanced level of the α-factor could then be detected by an increase in the area of non-growth in a lawn of responsive "a" yeast cells. In July and August, 1982, I conducted experiments which determined that yeast transformants containing MFα1 coding sequences

produced the most α-factor by growth inhibition tests. By August 27, 1982, I concluded that the 1.7 kb EcoRI (MFα1) fragment contained active α-factor pheromone gene. (Singh Exhibit 3, Notebook 1249, Bates No. 000071.)

24. On August 27, 1982, I concluded that p58 was a general vector for α-factor directed secretion that could be used with any gene which began with either an EcoRI restriction site next to the ATG (start) codon or a XbaI and EcoRI site next to the ATG codon. (Singh Exhibit 3, Notebook 1249, Bates Nos. 000072-000073.)

25. During the period September 1 to September 6, 1982, the plasmid DNAs from several *E. coli* transformants having the p58 α-factor-interferon constructions were tested by June Lugovoy using restriction enzyme digests. Three transformants appeared to have the appropriate insert. (Singh Exhibit 3, Notebook 1249, Bates No. 000074.)

26. During the period September 6 to September 11, 1982, June Lugovoy prepared DNA from two transformants containing the p58 plasmid. I instructed June Lugovoy to partially digest the plasmid DNA and to isolate a DNA fragment having the complete α-factor prepro/spacer sequence and the IFN-D sequence. This fragment was ligated into the yeast plasmid YEp9PT. (Singh Exhibit 3, Notebook 1249, Bates No. 000075). The resulting plasmid was designated p60. *E.coli* cells were

transformed with the ligation mixture and transformants having the plasmid p60 were identified. (Singh Exhibit 3, Notebook 1249, Bates No. 000075.) The Notebook of June Lugovoy shows the restriction map of p60. (Singh Exhibit 20, Notebook 861, Bates No. 000418.)

27. From September 12, 1982 to September 13, 1982, I transformed *S. cerevisiae* Pep4 cells with the plasmid p60 and obtained a number of transformants. (Singh Exhibit 3, Notebook, 1249, Bates Nos. 000075-000076.) I signed up with the Assay Group to conduct bioassays for interferon expression in the transformed yeast cells.

28. During the period September 14 to September 16, 1982, bioassays of the level of interferon in each of the yeast p60 transformant cultures were performed. (Singh Exhibit 3, Notebook 1249, Bates No. 000077-000080.) This bioassay tests the interferon activity of yeast extracts by comparing the activity of the yeast extracts to the activity of interferon standards using a vesicular stomatitis virus challenge of bovine kidney tissue culture cells. In the bioassay, the active interferon in the yeast extracts will prevent killing of the cells by the virus. This bioassay was a routine test for interferon activity. The interferon standard used was NIH leukocyte interferon standard G-203-901-527. The calculations done to determine the amount of interferon

secreted into the medium are shown as Singh Exhibit 3, Notebook 1249, Bates No. 000081.

29. By September 20, 1982, I had provided the *S. cerevisiae* Pep4-p60 strain to John Wulf. On September 27, 1982, I received the results of a fermentation run of the yeast containing the p60 plasmid from John Wulf. John Wulf had labeled this run WS1240. The results of this fermentation run show that there were 2×10^9 units of interferon per liter of medium. (Singh Exhibit 3, Notebook 1249, Bates No. 000085.)

30. Researchers at Genentech, Inc. had also been working on expression of DNA coding for human insulin-like growth factor (IGF). On September 25, 1982, I devised a method to insert the IGF structural gene into a yeast expression plasmid next to the α-factor prepro/spacer sequence (Singh Exhibit 3, Notebook 1249, Bates No. 000082). From September 25 to September 26, 1982, I isolated the 1140 bp sequence containing the α-factor prepro/spacer sequence from p58 and ligated it into the yeast vector YEp9T to make plasmid p65. (Singh Exhibit 20, Notebook 861, Bates No. 000419.) James Lee tested twenty-two colonies and found that twenty-one of these had the insert and ten had the correct orientation. (Singh Exhibit 3, Notebook 1249, Bate Nos. 000083-000084.) James Lee then inserted a 220 bp DNA fragment containing the IGF structural gene into the YEp9T vector with the α-factor sequence.

Expression and secretion of IGF1 was demonstrated by November 1, 1982. (Singh Exhibit 3, Notebook 1249, Bates No. 000101.)

31. On September 27, 1982, I designed a method of inserting the immune interferon gene into p65. (Singh Exhibit 3, Notebook 1249, Bates No. 000086.)

32. On October 1, 1982, I designed a method of inserting the tissue plasminogen activator gene (tPA) next to the prepro/spacer sequence of α-factor in a yeast plasmid. (Singh Exhibit 3, Notebook 1249, Bates No. 000087-000088.).

33. On October 1, 1982, Bill Kohr reported to me that the N-terminus of the processed IFN-D expressed by p60 yeast transformants contained 8 additional amino acids which corresponded to the four amino acids from the first spacer region of the prepro α-factor sequence immediately following the amino acids "lys arg", and four amino acids from the linker sequence at the beginning of the IFN-D sequence. These amino acids were "Glu Ala Glu Ala Leu Glu Phe Met." (Singh Exhibit No. 11, Bates Nos. 000201-000236)

34. After receiving this information from Bill Kohr, on or about October 1, 1982, I realized that I would need to design a yeast expression vector in which the codons coding for the four unwanted amino acids from the spacer region of the

prepro/spacer sequence of the α-factor gene and the four unwanted amino acids of the linker sequence at the beginning of the IFN sequence were deleted. Such a vector would allow expression of any protein in a manner so that it began at the correct N-terminal amino acid when the codon for the first amino acid in the gene was placed next to the codon for the Arg amino acid in the α-factor prepro/spacer sequence. On or about October 1, 1982, I told this idea to a number of people including Ron Hitzeman, John Wulf and Bill Kohr.

35. On October 5, 1982, while I decided how to construct a plasmid to delete the unwanted codons, I designed constructions using the calf rennin gene and α-factor promoter and complete prepro/spacer sequences in a yeast plasmid. (Singh Exhibit 3, Notebook 1249, Bates No. 000089.)

36. On October 5, 1982, I received the results from a protein gel which J. Perry had run on the media from the fermentation of yeast cells containing the p60 plasmid. These gels showed that the yeast media had a protein which migrated at the same rate as IFN-D in the gel. (Singh Exhibit 3, Notebook 1249, Bates No. 000089.)

37. During the period from October 6 to November 5, 1982, I conducted a number of restriction digests and ligations to construct a plasmid having the tPA gene next to the α-factor prepro sequence by the method as described above in paragraph

32. (Singh Exhibit 3, Notebook 1249, Bates Nos. 000090-92, 000094, 000099-0000100, 000102-000103.)

38. On October 12, 1982, I instructed June Lugovoy to perform ligations with vector p65 and EcoRI fragments of various genes, including γ-interferon, insulin-like growth factor (IGF), and human serum albumin (HSA), which were obtained from other scientists at Genentech, Inc. (Singh Exhibit 3, Notebook 1249, Bates No. 000092.) All of these ligations would place the gene next to the complete prepro/spacer sequence of α-factor. These ligation mixtures were used to transform *E.coli* 294 and miniscreens were performed on October 13, 1982. (Singh Exhibit 3, Notebook 1249, Bates No. 000093.)

39. On October 15, 1982, I devised a plan to construct a yeast expression plasmid having the γ-IFN gene joined to the DNA sequence in the α-factor prepro/spacer sequence immediately after the codons coding for the amino acids lys arg. This construct would eliminate the codons from the spacer region of the α-factor prepro/spacer which coded for the amino acids which were not removed during processing. On October 15, 1982, I wrote this into my Notebook 1249 at pages 46-49. (Singh Exhibit 3, Bates Nos. 000095-000098.) In order to construct the yeast plasmid having the γ-IFN gene, I needed to order a synthetic oligonucleotide complimentary to nucleotides 1201-1216 of the plus strand of α-factor. This was a 16mer with the

sequence TCTTTTATCCAAAGAT. The term 16mer refers to the number of nucleotides in the sequence. In this case there were 16 nucleotides. On November 1, 1982, I ordered a 16mer corresponding to the sequence TCTTTTATCCAAAGAT. The Synthetic DNA Request is shown at my Notebook 1249, at page 76. (Singh Exhibit 3, Bates No. 000125.) Peter Ng completed purification of this oligomer on November 16, 1982, as evidenced by his notes on the Synthetic DNA Specification. (Singh Exhibit 3, Notebook 1249, Bates No. 000125.) I also needed a DNA double stranded linker having the sequence

```
GATC TGT TAC TGT CAA
     ACA ATG ACA GTT CTG
```

While I designed this construct on October 15, 1982, I later decided to proceed by a different method.

40. On October 20, 1982, I instructed June Lugovoy to do transformations in *S. cerevisiae* Pep4 using plasmids having the complete prepro/spacer α-factor sequence and the genes for either HSA, IGF and γ-interferon which had been previously constructed. (Singh Exhibit 3, Notebook 1249, Bates No. 000099.)

41. On October 22, 1982 a memo was issued by Jim Swartz which indicated that the prepro α-factor-LIF-D plasmid in Pep 4-3 yeast had produced LIFD ("IFN-D") in the supernatant which comprised 40-50% of the total protein in the supernatant. This memo indicates that the LIFD in the supernatant consisted of mature

LIFD plus eight amino acids. All but the pertinent information has been blocked out of this exhibit. (Document GZ00051406-1409) (Singh Exhibit 18, Bates Nos. 000403-000406.)

42. On October 25, 1982, a Yeast Project Team meeting was held at which I discussed α-factor expression systems. J. Swartz and R. Hitzeman attended. A Summary of this meeting (Document GX0000687-691.) (Singh Exhibit 19, Bates Nos. 000407-000411.) shows that other genes had been tested with the α-factor system, including interferon, IGF, immune interferon, and HSA. The Summary also states that I was working on constructions that would allow cleavage following the lysine arginine of α-factor presequence to release mature protein with a correct N-terminal end. All but the pertinent information has been blocked out of this Summary.

43. On November 8, 1982, I transformed *E.coli* cells with the YEp13 vector having the tPA gene ligated to the complete α-factor prepro/spacer sequence constructed from October 6, 1982 to November 5, 1982 (paragraph 37). From November 11 to November 16, 1982, I digested the DNA from a number of transformants to determine if I had the correct plasmid construct (Singh Exhibit 3, Notebook 1249, Bates Nos. 000103-106). I determined I had the proper construct and labeled it p68. (Singh Exhibit 3, Notebook 1249, Bates No. 000106.) The Notebook

of June Lugovoy shows the restriction map of p68. (Singh Exhibit 20, Notebook 861, Bates No. 000422.) I gave the plasmid DNA to June Lugovoy to transform yeast.

44. On November 23, 1982, I tested the yeast A145 transformed with the plasmid p68 on fibrin plates for production of tPA. (Singh Exhibit 3, Notebook 1249, Bates No. 000107). On December 2, 1982, I obtained the results of my test. (Singh Exhibit 3, Notebook 1249, Bates No. 000113.) I also digested p56 and isolated a 1300 bp fragment which I sent to E. Chen for sequencing. (Singh Exhibit 3, Notebook 1249, Bates No. 000107.)

45. On November 24, 1982, I designed an MFα1-IFNα-1 junction sequence. The sequence currently at the junction between the α-factor prepro gene sequence and the IFND gene in plasmid p58 was

```
leu asp lys arg glu ala glu ala leu glu phe met cys asp leu pro
TTG GAT AAA AGA GAG GCT GAA GCT CTA GAA TTC ATG TGT GAT CTC CCT
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The sequence to be removed was

```
glu ala glu ala leu glu phe met
GAG GCT GAA GCT CTA GAA TTC ATG
```

In order to remove this sequence by oligonucleotide deletion mutagenesis, I needed to order a synthetic single-stranded DNA fragment having the sequence TTGGACAAGAGATGTGACTTGCCA. I requested this 24mer on November 24, 1982 then canceled the request the same day noting at Notebook 1249, page 59 (Singh

Exhibit 3, Bates No. 000108), that I would do this in a different way and without changing codons.

46. On November 29, 1982 I inoculated cultures with the tPA/A145 transformants. On December 2, 1982, I noted that seven transformants expressed intracellular tPA and two of those secreted tPA as well. (Singh Exhibit 3, Notebook 1249, Bates Nos. 000109, 000113.)

47. On December 1, 1982, I prepared a synthetic DNA request for a 24mer. This oligonucleotide was to be used for making the inframe deletion of the junction of α-factor pro sequence and interferon instead of the oligonucleotide discussed at paragraph 45. The 24mer requested was AGGGAGATCACATCTTTTATCCAA. A copy of this Synthetic DNA Request as well as a Synthetic DNA Specification form is shown at Notebook 1249, page 77 (Singh Exhibit 3, Bates No. 000126). As indicated on the Synthetic DNA Specifications, the purification of this 24mer was completed on December 20, 1982. (Singh Exhibit 3, Notebook 1249, Bates No. 000126.)

48. On December 4, 1982, I entered into my Notebook the results of the radioimmunoassay of the level of tPA produced by the transformants having the tPA gene and the complete α-factor prepro/spacer sequence on plasmid p68. This test

shows the expression and secretion of tPA using α-factor-tPA constructs. (Singh Exhibit 3, Notebook 1249, Bates No. 000114.)

49. From December 5, 1982 to December 27, 1982, I designed and constructed another tPA expression plasmid. This plasmid could be used in the Pep 4 strain of yeast (which is a trp auxotroph) because the plasmid contained the TRP1 gene. (Singh Exhibit 3, Notebook 1249, Bates Nos. 000116-000117, 000119-000123, 000127-000128.)

50. On December 14, 1982, I presented my results to the Research Review Group (RRG). Yeast secretion was discussed by myself and Ronald Hitzeman. Singh Exhibit 25 (Bates Nos. 000455-000487) includes a summary of the meeting and the documents I presented at the Meeting. In this Exhibit all but the pertinent information has been blocked out. At this meeting I described the α-factor gene organization and its structure including the spacer having the sequence lys arg followed by glu ala's. I also described that my future work would include removal of sequences from the interferon D expression plasmid with site directed deletion mutagenesis and construction of expression plasmids with a restriction site following the α-factor prepro sequence. (Singh Exhibit 25, Bates Nos. 000455-000487.)

51. On December 15, 1982, I received the results of a partial EcoRI digest of p58 prepared by June Lugovoy and isolated the 1720 base pair fragment containing the complete α-factor promoter and prepro/spacer interferon-D gene as shown at Notebook 1249, page 73. (Singh Exhibit 3, Bates No. 000122.)

52. On December 18, 1982, I isolated the 1800 base pair complete α-factor promoter prepro/spacer-interferon D sequence from p60 and inserted the fragment into the plasmid M13mp8 for deletion mutagenesis, as shown at Notebook 1249, page 75. (Singh Exhibit 3, Bates No. 00124.)

53. From December 31, 1982 to January 3, 1983, I transformed *E.coli* with the ligation mixture having the tPA expression vector allowing trp selection. I also transformed *E.coli* with the M13mp8 ligation mixtures having the α-factor-IFND sequence. I obtained a number of transformants as shown at Notebook 1249, pages 80 and 81. (Singh Exhibit 3, Bates Nos. 000129-000130.)

54. On January 5, 1983, I pasted the restriction map of M13mp8 into my Notebook 1249, page 82. (Singh Exhibit 3, Bates No. 000131.) On January 6, 1983, I digested the transformants I had obtained with a number of restriction enzymes and determined that three recombinant M13mp8 plasmids containing the EcoRI insert

having the spacer sequence were obtained as indicated at Notebook 1249, page 83. (Singh Exhibit 3, Bates No. 000132.)

55. The results of a December 20, 1982 fermentation of *S. cerevisiae* Pep 4-3 having a plasmid with α-factor linked to the HSA gene were received by me from John Wulf on either January 9 or January 10, 1983, as shown in Notebook 1249, page 84. (Singh Exhibit 3, Notebook 1249, Bates No. 000133.) John Wulf had labeled this fermentation run WS141Q.

56. On January 19, 1983, I requested the synthesis of two 15 mer α-factor sequences, #3 - TGCCAGGAGCATCAA and #4 - ATTGCCAGCATTGCT. The reference number for #3 was V1535-1 and for #4 was V1535-2. Column 7 of Peter Ng's log book shows that Peter Ng completed the purification of both sequences on January 20, 1983. (Singh Exhibit 6, Log Book, Bates Nos. 000187-000188; Singh Exhibit 8, Notebook 1507, Bates No. 000195.)

57. On or about January 20, 1983, I received from John Wulf the results of a January 12, 1983 fermentation of yeast having a plasmid with α-factor linked to the bovine interferon gene as shown in Notebook 1249, page 95. (Singh Exhibit 3, Notebook 1249, Bates No. 000144.)

58. From January 21, 1983 to January 27, 1983, I conducted deletion mutagenesis of the α-factor spacer-IFN-D sequence cloned into M13mp8 using the synthetic 24 mer ordered on December 15, 1982 (Singh Exhibit 3, Notebook 1249, Bates No. 000136-000139). During the period 1982 to 1983 site directed deletion mutagenesis was a known technique. As set forth in Sambrook, et al. "Molecular Cloning" 2nd Edition (1989) at pages 15.51 and 15.52 (Singh Exhibit 36, Bates Nos.000564-000566), this technique was known in the early 1970's and had developed into an established methodology by 1982. Seven of the deletion mutant plasmids were chosen for sequencing and C-tracking on January 31, 1983.

59. During the period February 8 to February 10, 1983, I C-tracked and/or sequenced the mutants prepared as described above in paragraph 58. I noted that I loaded the gel with Dennis. By February 10, 1983, the sequence of one of the mutants at the junction of the α-factor prepro sequence with the interferon gene had been determined and indicated that the sequence coding for the 8 unwanted amino acids had been deleted. These results are shown at Notebook 1576, pages 1-4. (Singh Exhibit 4, Notebook 1576, Bates Nos. 000146, 000148-000151.) On February 17, 1983, I inserted the gel of the C-track experiments into my Notebook 1576 at page 5 (Singh Exhibit 4, Notebook 1576, Bates No. 000152), and on February 19, 1983, I noted in Notebook 1576, at page 6 (Singh Exhibit 4, Notebook 1576, Bates Nos.

000153) that I would sequence one of the three mutant clones that appeared to have the desired deletion on the basis of the C-tracking data.

60. On February 25, 1983, I isolated the EcoRI fragment having the deletion. (Singh Exhibit 4, Notebook 1576, Bates No. 000156.) This fragment was inserted into the plasmid YEp9T to create p76. J. Lugovoy's Notebook shows the restriction map of p76. (Singh Exhibit 20, Notebook 861, Bates No. 000425.)

61. By February 27, 1983 a similar expression plasmid, p77, was constructed by inserting the EcoRI fragment with the desired deletion into plasmid p70. The restriction map of p77 is on page 12 of my Notebook 1576. (Singh Exhibit 4, Notebook 1576, Bates No. 000159.)

62. From February 27, 1983 to March 9, 1983, June Lugovoy transformed yeast strain 20B-12 with plasmids p76 and p77. She isolated the yeast transformants, prepared extracts of yeast and then submitted the culture supernatants and the cell extracts to the Assay Group to determine expression and secretion of interferon by the transformants. (Singh Exhibit 4, Notebook 1576, Bates Nos. 000160 and 000162)

63. On March 10, 1983, I reported in my Notebook the results of radioimmunoassays for tPA from yeast having either plasmid p72 or p68, as shown in Notebook 1576, page 14. (Singh Exhibit 4, Notebook 1576, Bates No. 000161.)

64. On March 11, 1983, I recorded the results of interferon bioassays of fermentations of yeast having plasmid p76 and p77. The interferon assays were performed on March 9, 1983. I determined that yeast having plasmid p76 or p77 secreted interferon into the supernatant. (Singh Exhibit 22, Notebook 1563, Bates No. 000435; Singh Exhibit 4, Notebook 1576, Bates No. 000162.)

65. In order to determine whether the IFN-D protein after processing had the correct N-terminal sequence, I needed to have the product expressed by p76 sequenced. On March 14, 1983, I dialyzed the medium from a p76/Pep 4 fermentation for amino acid sequencing. I pasted into my Notebook the procedure from Jeanne Perry (Singh Exhibit 4, Notebook 1576, Bates No. 000163). I previously had sent the yeast strain having the p76 plasmid to Fermentation. (Singh Exhibit 27, Bates No. 000502-000508)

66. On April 21, 1983, in order to separate the α-factor promoter from the α-factor prepro sequence so that the promoter could also be used independently of secretion signals to direct the expression of various genes, I requested the synthesis of

a 26 mer α-factor promoter sequence, TATCGATTTCATACACAATATAAACT; and a 31 mer α-factor promoter sequence, GATAGCTAAAGTATGTGTTATATTTGAGATC. The reference number for the 26 mer sequence was D1680-13A. The reference number for the 31 mer sequence was D1680-13B. (Singh Exhibit 6, Log Book, Bates No. 000189, Singh Exhibit 8, Notebook 1507, Bates No. 000196.)

67. On May 6, 1983, in order to separate the α-factor prepro sequence from the α-factor promoter so that the α-factor prepro sequence could be used independently, I requested the synthesis of a 24 mer α-factor signal #2 sequence, GTGAAAATAGATGGGAATCTCATG. The reference number for the 24 mer sequence was D1680-15B. (Singh Exhibit 6, Log Book, Bates No. 000189; Singh Exhibit 9, Notebook 1798, Bates No. 000198)

68. On May 7, 1983, I received the results of a May 5, 1983 assay for interferon on the interferon α-1 samples from the p76/20B-12 fermentation performed by J. Wulf. Jeanne Perry isolated the interferon from the fermentation media. A mammalian cell lysis bioassay was conducted on the isolated leukocyte interferon from the fermentation samples, as shown in my Notebook 1576 at page 26. (Singh Exhibit 4, Notebook 1576, Bates No. 000173.) The yeast having the p76 plasmid were producing interferon which was active.

69. On May 11, 1983, in order to separate the α-factor prepro sequence from the α-factor promoter so that the α-factor prepro sequence could be used independently, I requested the synthesis of a 32 mer α-factor signal #1 sequence, AATTCATGAGATTCCCATCTATTTTCACTGCA. The reference number for the 32 mer sequence was D1680-15A. (Singh Exhibit 6, Log Book, Bates No. 000189; Singh Exhibit 9, Notebook 1798, Bates No. 000198.)

70. On or about June 2, 1983, Bill Kohr told me the sequence of the interferon secreted from yeast cells having the α-factor with the deletion linked to the IFN gene (p76). Mature interferon without the preceding eight amino acids was indicated because the N-terminal sequence was Cys Asp Leu Pro Glu Trp His Ser Leu. This was consistent with the data shown in Singh Exhibit 12, Bates No. 000237-000256. This confirmed that the plasmid p76 which did not have the codons for amino acids glu ala glu ala after the lys arg codons in the spacer could be used to produce correctly processed proteins in yeast.

71. I further declare that all statements made of my own knowledge are true, and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false

Interferon Assays

BK.-258 → B.K. 302

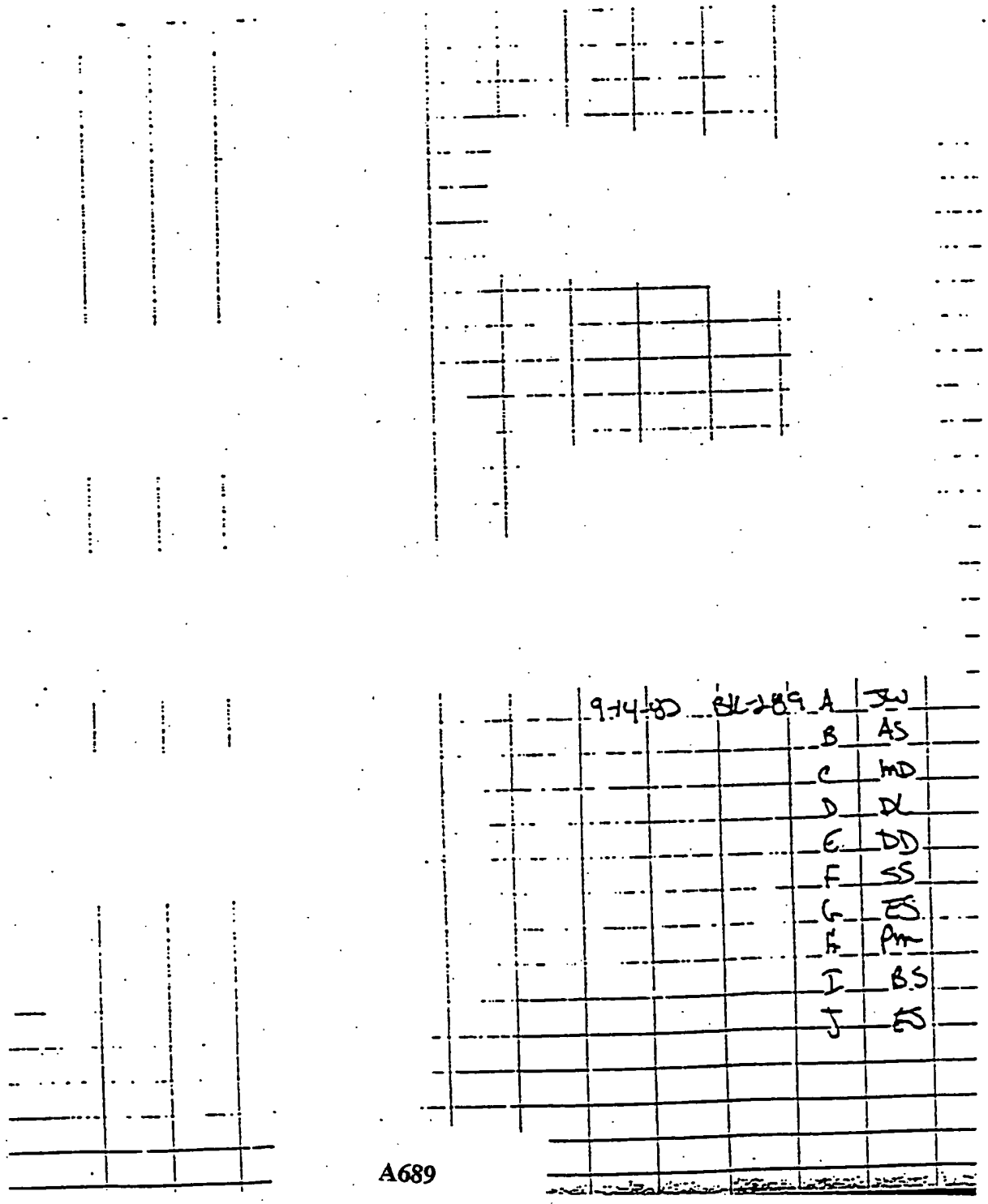
Singh Exhibit 39
Singh v. Brake
Interference No. 102,728

000583

8-2-82 → 10-1-82

000584

Date Assay Facility Date Assay Facility



000585

Date	Assy #	For whom
9-15-82	BK240 A	MD
	B	PM
	C	SS
	D	ES
	E	LF
	F	AS
9-16-82	BK291 A	DD
	B	DL
	C	PM
	D	SS
	E	MD
	F	JW
	G	AS

INTERFERON BIOASSAY DATA SHEET

000586

Assay Number: BK-289

Date Initiated: 9-14-81

Cells: Line and passage no.: MDBK ATC P-124
Cultivated by: TP
Medium dated:

Samples loaded by: LF, Km

5. Dilutions by: LF Km

6. Standards used and dates prepared:

LIF C Thaw Date: Well No.: 83 Conv. Fact:
LIF R Thaw Date: Well No.: F85 Conv. Fact:
LIF Thaw Date: Well No.: Conv. Fact:
FIF Thaw Date: Well No.: Conv. Fact:
IFN Thaw Date: Well No.: Conv. Fact:

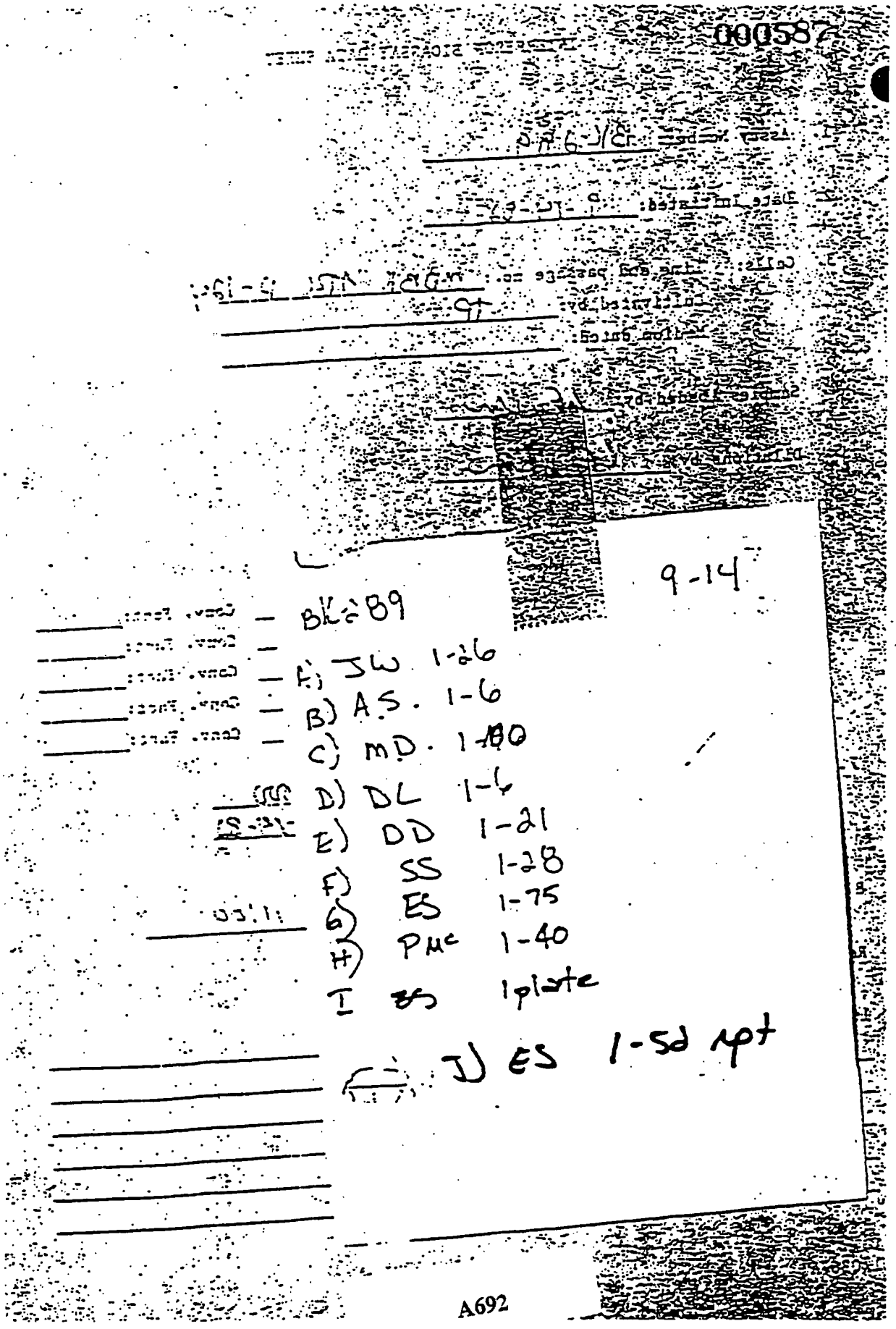
7. Virus: Lot No: USV#133 Dilution: 1:300
Diluted by: Km Thaw Date: 9-14-81
Time added: 9:30

8. Stained by: LF Time stained: 11:00

Read by: LF

10. Comments, Problems:

1/0367A


9-14
A) JW 1-26
B) A.S. 1-6
C) MD. 1-10
D) DL 1-4
E) DD 1-21
F) SS 1-28
G) ES 1-75
H) PMC 1-40
I ES 1plate
J ES 1-52 apt

000588

GENENTECH
INTERFERON BIOASSAY

ASSAY NUMBER: BK-2898
DATE OF ASSAY: 9/14/82

SAMPLE VOLUME=20
STANDARD: LIF-A
ACTIVITY OF STANDARD (U/ML)=1500
END POINT WELL: 8.5

DILUTION AT ENDPOINT=1920
CONVERSION FACTOR=.78125

NAME OF ASSAYER: ARJUN SINGH

SAMPLE#	END PT.	UNITS/ML	COMMENTS
1	>11	>8000	
2	>11	>8000	
3	>11	>8000	
4	>11	>8000	
5	9.5	3000	
6	7.5	750	

END OF ASSAY

INTERFERON BIOASSAY DATA SHEET

000589

1. Assay Number: BK-290

2. Date Initiated: 9-15-81

3. Cells: Line and passage no.: MDBK ATC P-110
Cultivated by: TP
Medium dated: 9/3/81 [illegible]

4. Samples loaded by: [illegible]

5. Dilutions by: LF, TP

6. Standards used and dates prepared:

LIF	C	Thaw Date:	9-15	Well No.:		Conv. Fact:	
LIF	A	Thaw Date:	9-15	Well No.:		Conv. Fact:	
LIF		Thaw Date:		Well No.:		Conv. Fact:	
FIF		Thaw Date:		Well No.:		Conv. Fact:	
IFN		Thaw Date:		Well No.:		Conv. Fact:	

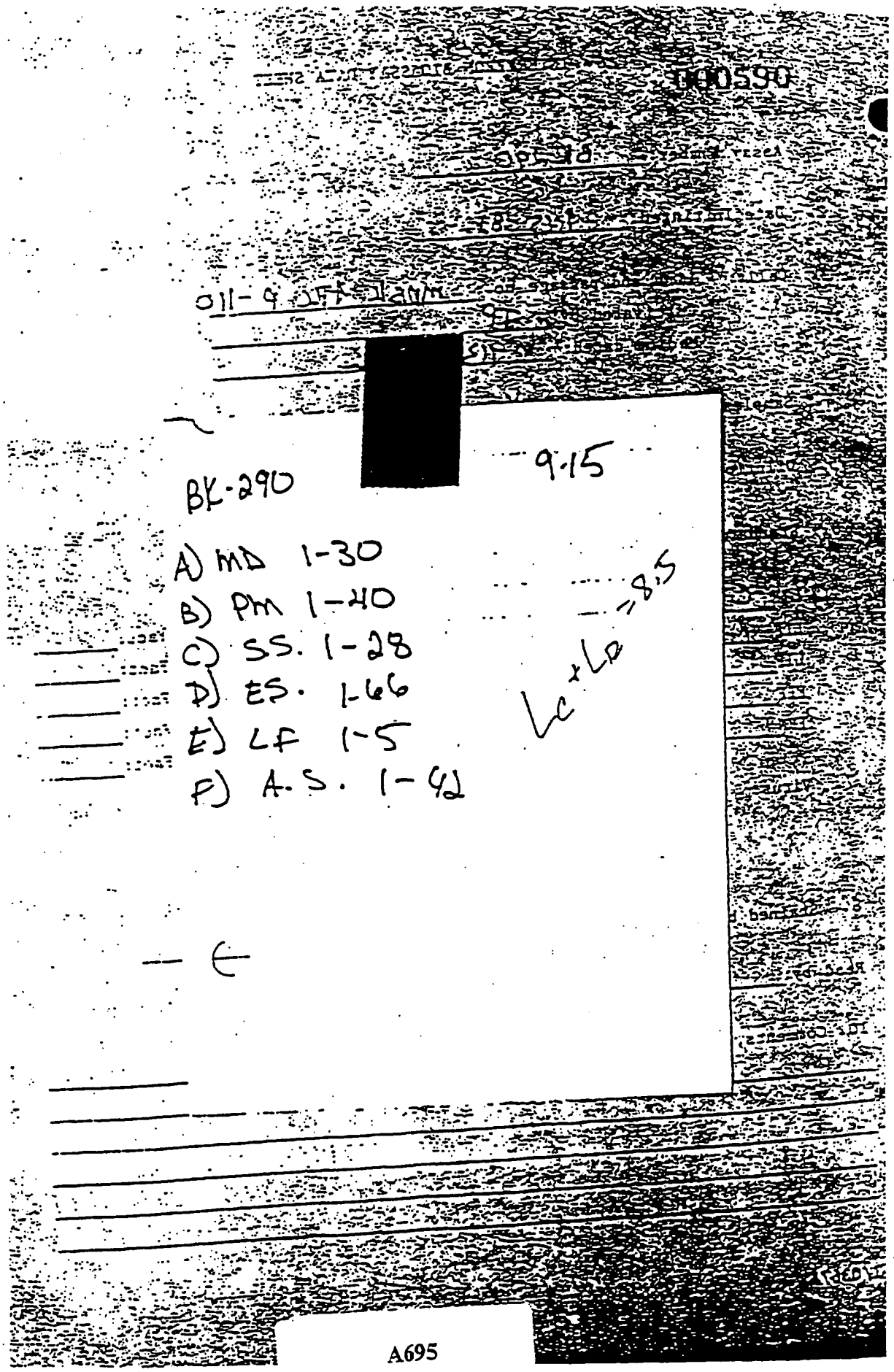
7. Virus: Lot No: [illegible] Dilution: 1:3000
Diluted by: [illegible] Thaw Date: 9-15
Time added:

8. Stained by: Time stained:

Read by:

10. Comments, Problems:

1/0367A



BK-290

A) MD 1-30
B) PM 1-40
C) SS. 1-28
D) ES. 1-66
E) LF 1-5
F) A.S. 1-42

9.15

$LC + LD = 8.5$

GENENTECH
INTERFERON BIOASSAY

000591

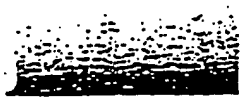
ASSAY NUMBER: BK-290F
DATE OF ASSAY: 9/15/82

SAMPLE VOLUME=20
STANDARD: LIF-A
ACTIVITY OF STANDARD (U/ML)=1500
END POINT WELL: 8.5

DILUTION AT ENDPOINT=1920
CONVERSION FACTOR=.78125

NAME OF ASSAYER: ARJUN SINGH

SAMPLE#	END PT.	UNITS/ML	COMMENTS
1	>11	>8000	
2	>11	>8000	
3	>11	>8000	
4	>11	>8000	
5	>11	>8000	
6	>11	>8000	
7	>11	>8000	
8	>11	>8000	
9	9.5	3000	
10	7.5	750	
11	>11	>8000	TOXIC TO WELL 3
12	>11	>8000	TOXIC TO WELL 3
13	>11	>8000	TOXIC TO WELL 3
14	>11	>8000	TOXIC TO WELL 3
15	>11	>8000	TOXIC TO WELL 3
16	>11	>8000	TOXIC TO WELL 3
17	>11	>8000	TOXIC TO WELL 3
18	>11	>8000	TOXIC TO WELL 3
19	>11	>8000	TOXIC TO WELL 3
20	>11	>8000	TOXIC TO WELL 3
21	10.5	6000	
22	>11	>8000	
23	10.5	6000	
24	9.5	3000	
25	10.5	6000	TOXIC TO WELL 1.
26	>11	>8000	
27	>11	>8000	
28	>11	>8000	
29	11	8000	
30	9.5	3000	
31	>11	>8000	
32	>11	>8000	
33	>11	>8000	
34	>11	>8000	
35	>11	>8000	
36	>11	>8000	
37	>11	>8000	
38	>11	>8000	
39	9	2000	
40	8	1000	
41	3.5	47	



ASSAYER: ARJUN SINGH DATE :9/15/82 PAGE :2

SAMPLE#	END PT.	UNITS/ML	COMMENTS
42	<1	<8	

END OF ASSAY

000592

INTERFERON BIOASSAY DATA SHEET

000593

Assay Number: BI-291

Date Initiated: 9-16-83

3. Cells: Line and passage no.: MDBK ATCC P-110

Cultivated by: TP

Medium dated: 91383 [illegible]

4. Samples loaded by: [illegible]

5. Dilutions by: TP, LF

6. Standards used and dates prepared:

LIF C	Thaw Date: 9/13	Well No.: 85	Conv. Fact: 78
LIF R	Thaw Date: 9/15	Well No.: 85	Conv. Fact: 78
LIF	Thaw Date:	Well No.:	Conv. Fact:
LIF	Thaw Date:	Well No.:	Conv. Fact:
FIF	Thaw Date:	Well No.:	Conv. Fact:
IFN	Thaw Date:		

7. Virus: Lot No. VSV #1 33 Dilution: 1:300
Diluted by: Thaw Date: 9-16-83
Time added:

8. Stained by: LF Time stained:

Read by: LF

10. Comments, Problems:

1/0367A

A698

000594

BK-291 9-16

A) DD 1-24

B) DL 1-6

C) PM 1-40

D) SS 1-20

E) MD 1-30

F) JW 1-24

G) AS 1-46

GENENTECH
INTERFERON BIOASSAY

000595

ASSAY NUMBER: BK-291G
DATE OF ASSAY: 9/16/82

SAMPLE VOLUME=20
STANDARD: LIF-A
ACTIVITY OF STANDARD (U/ML)=1500
END POINT WELL: 8.5

DILUTION AT ENDPOINT=1920
CONVERSION FACTOR=.78125

NAME OF ASSAYER: ARJUN SINGH

SAMPLE#	END PT.	UNITS/ML	COMMENTS
1	>11	>8000	TOXIC TO WELL 1
2	>11	>8000	
3	10	4000	
4	9.5	3000	
5	>11	>8000	
6	>11	>8000	
7	>11	>8000	
8	>11	>8000	TOXIC TO WELL 1
9	6.5	375	
10	4.5	94	
11	6.5	375	
12	7.5	750	
13	5.5	188	
14	4.5	94	
15	6.5	375	
16	7.5	750	
17	7.5	750	
18	8.5	1500	
19	3	31	
20	1.5	12	
21	8	1000	
22	8.5	1500	
23	8	1000	
24	7	500	
25	8.5	1500	
26	8.5	1500	
27	9	2000	
28	9	2000	
~~29~~	~~7~~	~~500~~	
29	7.5	750	
30	6.5	375	
31	>11	>8000	
32	>11	>8000	
33	>11	>8000	
34	>11	>8000	
35	>11	>8000	
36	>11	>8000	
37	>11	>8000	
38	>11	>8000	
39	7.5	750	
40	6.5	375	

ASSAYER: ARJUN SINGH DATE :9/16/92 PAGE :2

SAMPLE#	END PT.	UNITS/ML	COMMENTS
41	7.5	750	
42	8.5	1500	
43	6.5	375	
44	6.5	375	
45	8	1000	
46	6	250	

END OF ASSAY

000596

the sequence determination, and it was my practice to date the standard form with the date of the first sequencing run, as determined from the output tape.

6. Although neither of the completed standard forms described in the following paragraphs 7. and 8. is signed, I recognize the handwriting as my own, and acknowledge that it was my practice to complete these forms on or about the day that I determined the amino acid sequence of a protein. Thus, if a standard form is dated October 1, 1982, it means that I completed the amino acid sequence determination for that protein on or about October 1, 1982. It was also my practice to report the results of my sequence determination to the person who had requested the determination within a few days of obtaining my results.

7. On or about October 1, 1982 I reported to Arjun Singh the N-terminal sequence of the processed IFN-D expressed by p60 yeast transformants. It contained 8 additional amino acids which correspond to the four amino acids of the linker region of the α-factor prepro/linker region and the four amino acids at the beginning of the IFN-D gene. These amino acids were Glu Ala Glu Ala Leu Glu Phe Met. I know this, because this is the amino acid sequence indicated on my standard form dated October 1, 1982 found in Singh Exhibit 11, Bates Nos. 000201-236. This standard form is accompanied by the output from the sequencing runs, also dated October 1, 1982.

8. On or about the time I reported my October 1, 1982 results to Arjun, I discussed these results with him. I remember that Arjun was excited about the results, since it was clearly interferon. I remember that Arjun was expecting the additional Glu Ala sequences, and I remember talking with Arjun about strategies to modify the DNA to eliminate the Glu Ala's, although I do not remember any details of how he proposed to do this.

9. On or about June 2, 1983 I reported to Arjun Singh the N-terminal amino acid sequence of the interferon secreted from yeast cells having the α-factor with the deletion linked to the IFN gene (p76). Mature interferon without the preceding eight amino acids was indicated. I know this, because the N-terminal sequence indicated on the standard form dated June 2, 1983 was Cys Asp Leu Pro Glu Trp His Ser Leu. This standard form is dated June 2, 1983 and is accompanied in Singh Exhibit 12, Bates Nos. 000237-256, by the output from the sequencing runs, which are dated June 2 and 3, 1983.

10. When I reported the June 2, 1983 results to Arjun, I remember discussing with Arjun that his experiments removing the DNA encoding the additional amino acids had been successful so that mature interferon having the correct N-terminal sequence was secreted.

11. I further declare that all statements made of my own knowledge are

Sample # UFD-1 Sequencer 811 Run Date 10-1-82

?-Factor, med. Amount applied 50λ/10+100 In sol. H2O (?)

Met. of appl. 450λ PBS 6-LCy Program notes ? 3.P ?

Temp 9.0

..tion Injected	Cycle #	Amino acid Id.	Peak area ÷ 10⁶	calc. # of nmoles	calc. # of total nm in sample seq.	Carry over amino acid	Area of carry over ÷ 10⁶	# of nm or % carry over	Other note or minor sec. or amino acids
	1	Glu	4.3		19				many ? peak.
	2	[Ala]							with ?
	3	Glu	3.7		7.39				
	4	Ala	7.2		19.7/16				plus some ?
	5	Leu	3.6		10				
	6	Glu	2.8		8.2				
	7	Phe	5.2*		16				* plus other
	8	Met	2.0		6.5				
	9	[Cys]	—		—				
	10	Asp	2.5		7.1/4.4				
	11	Leu	3.07		9.1/3.4				
	12	Pro	1.6		4.4/1.7				
	13	Glu	2.2		7.2/4.9				
	14	Thr	.8		2.1/.4				
	15	[His]	—						
	16	[Ser]	—						
	17	[Leu]	1.8		5.4/4.3				
	18	[Asp]	1.7		4.7/4.2				
	19	[Asn]	1.0		2.9/1.5				

000201

Singh Exhibit 11
Singh v. Brake
Interference No. 102,728

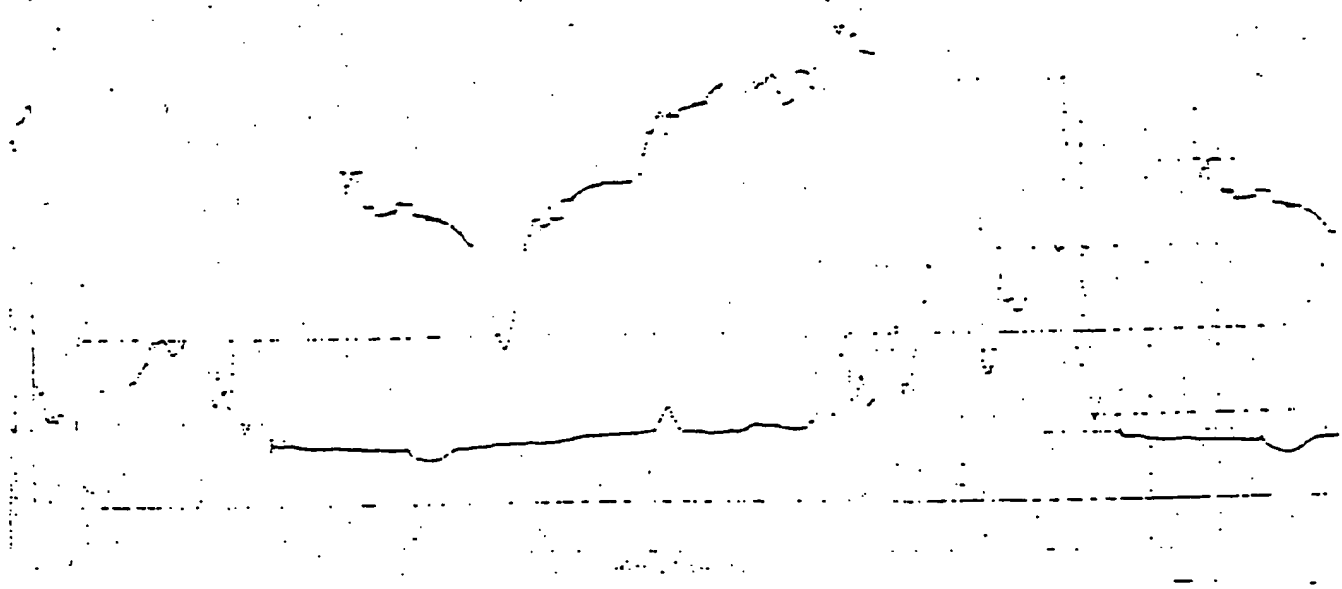
10-1-82

LIFD - Yeast

2 Factor direct Sm media

~25 mg

Cy 1-19



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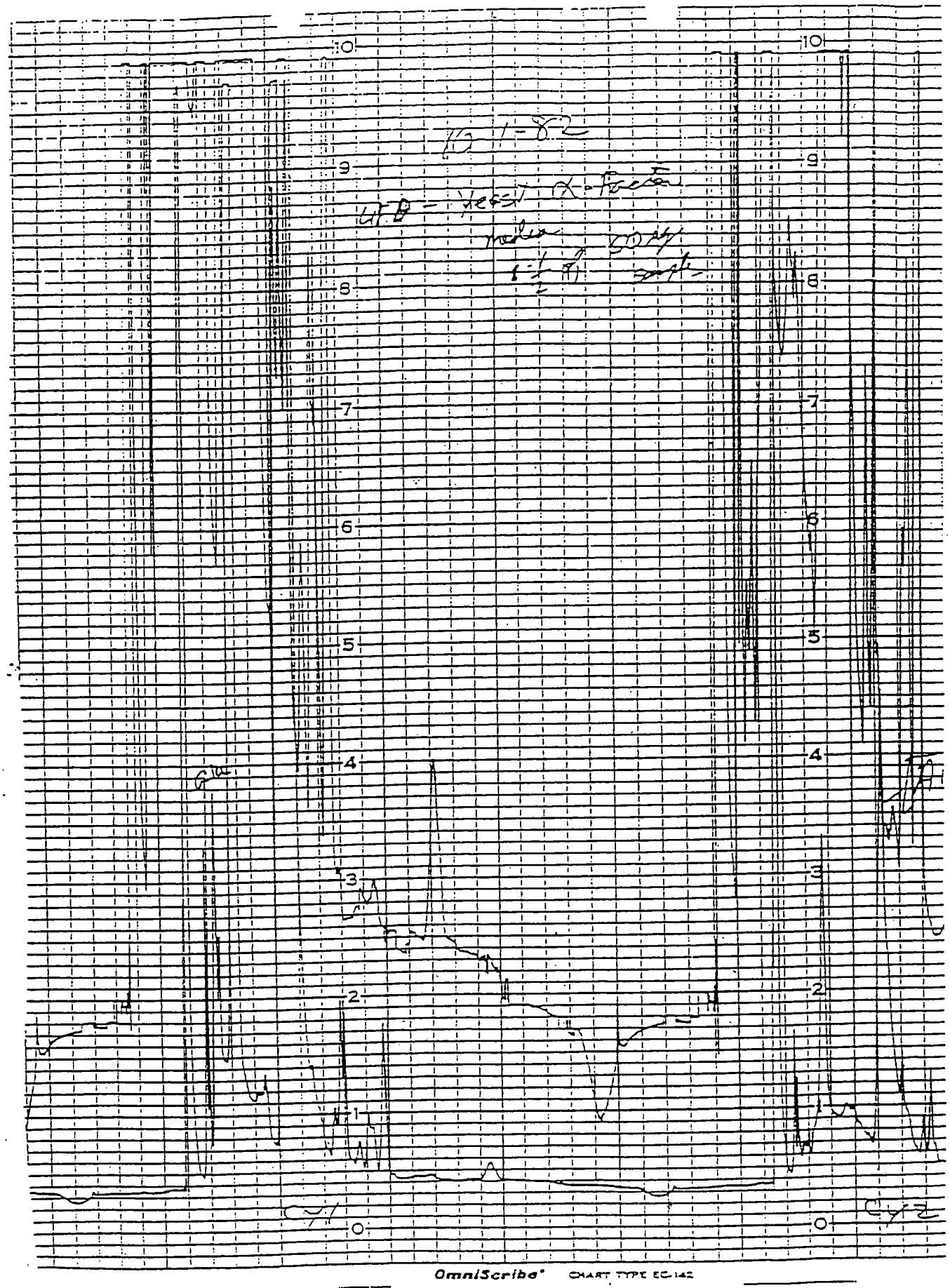

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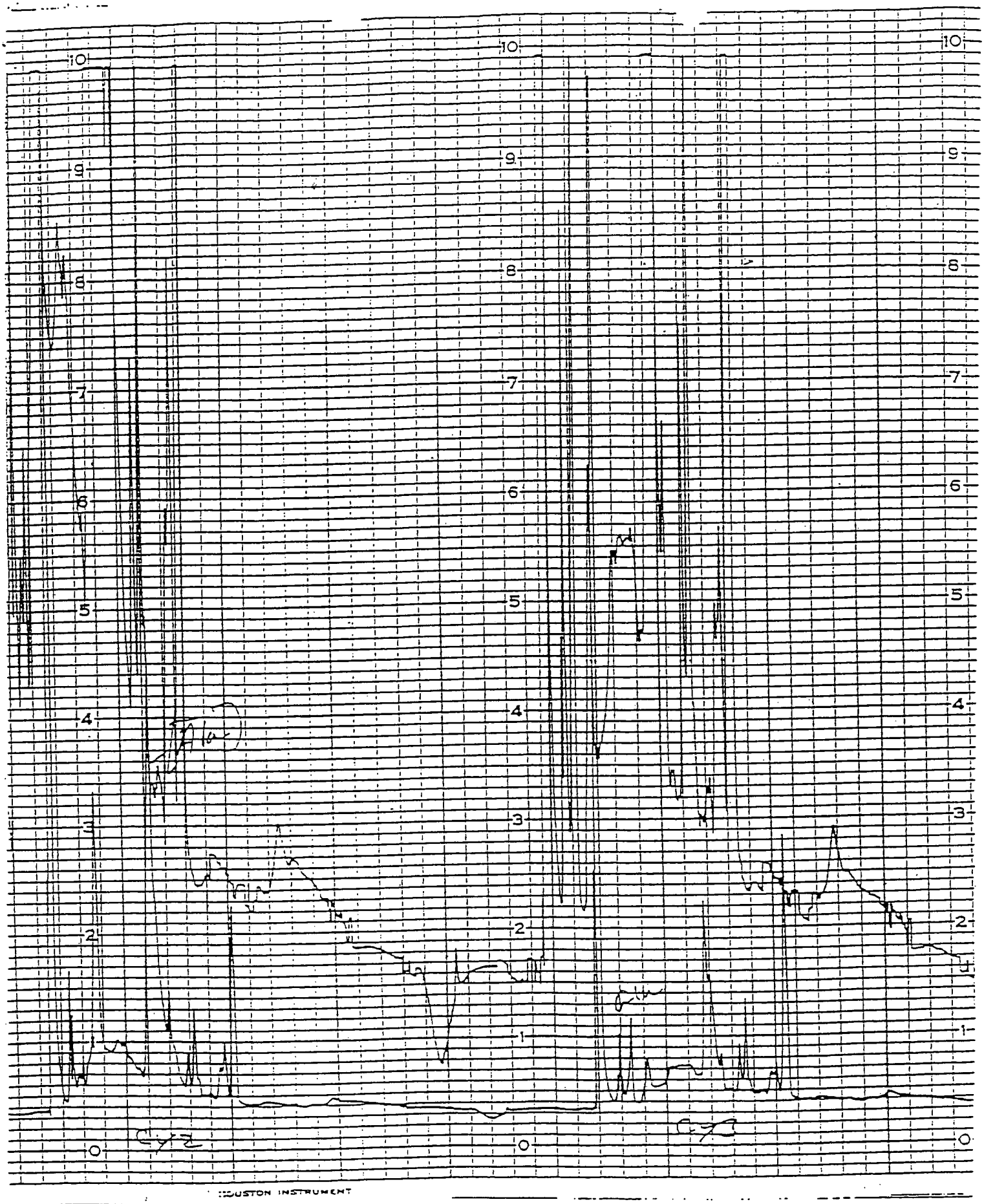

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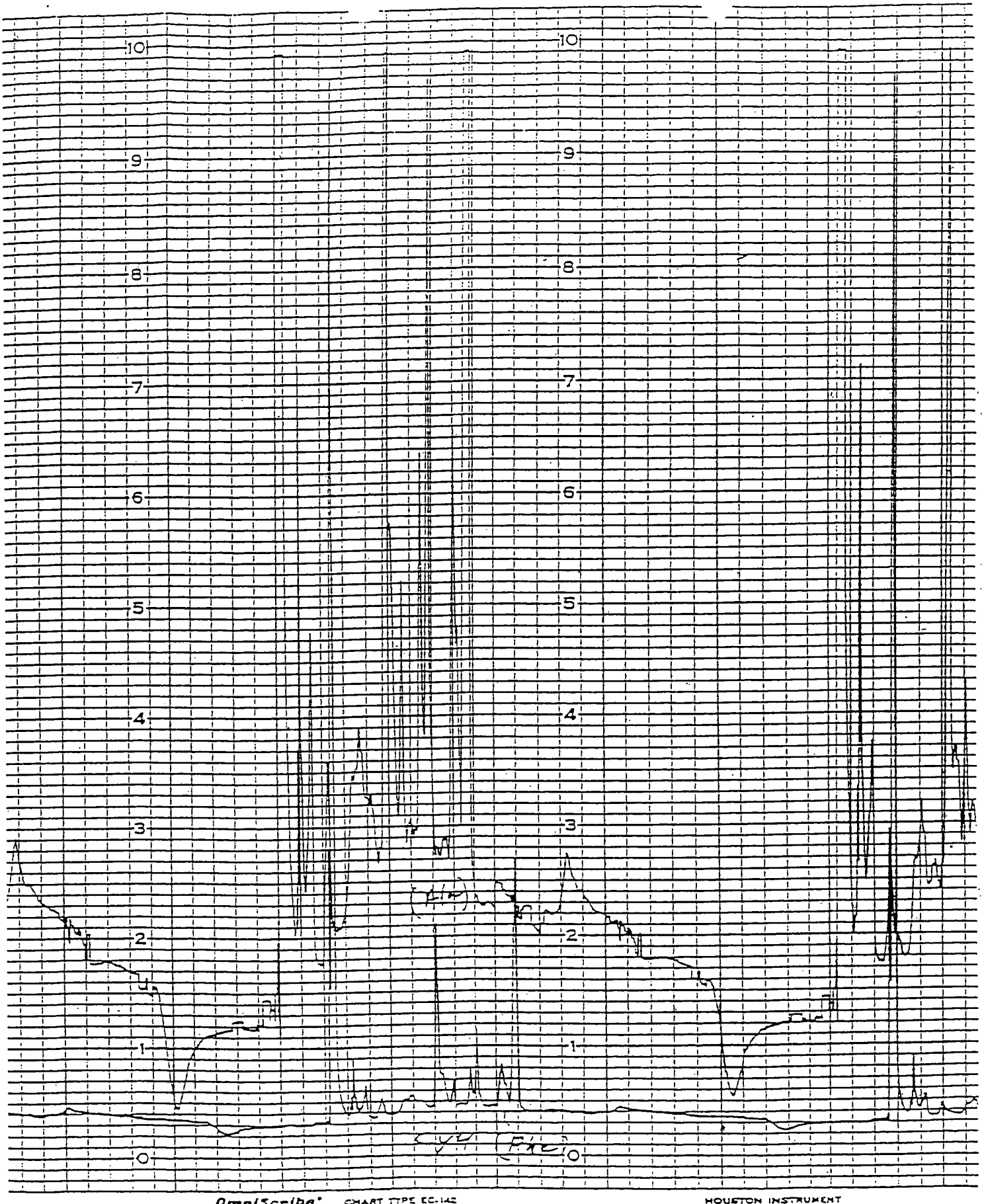
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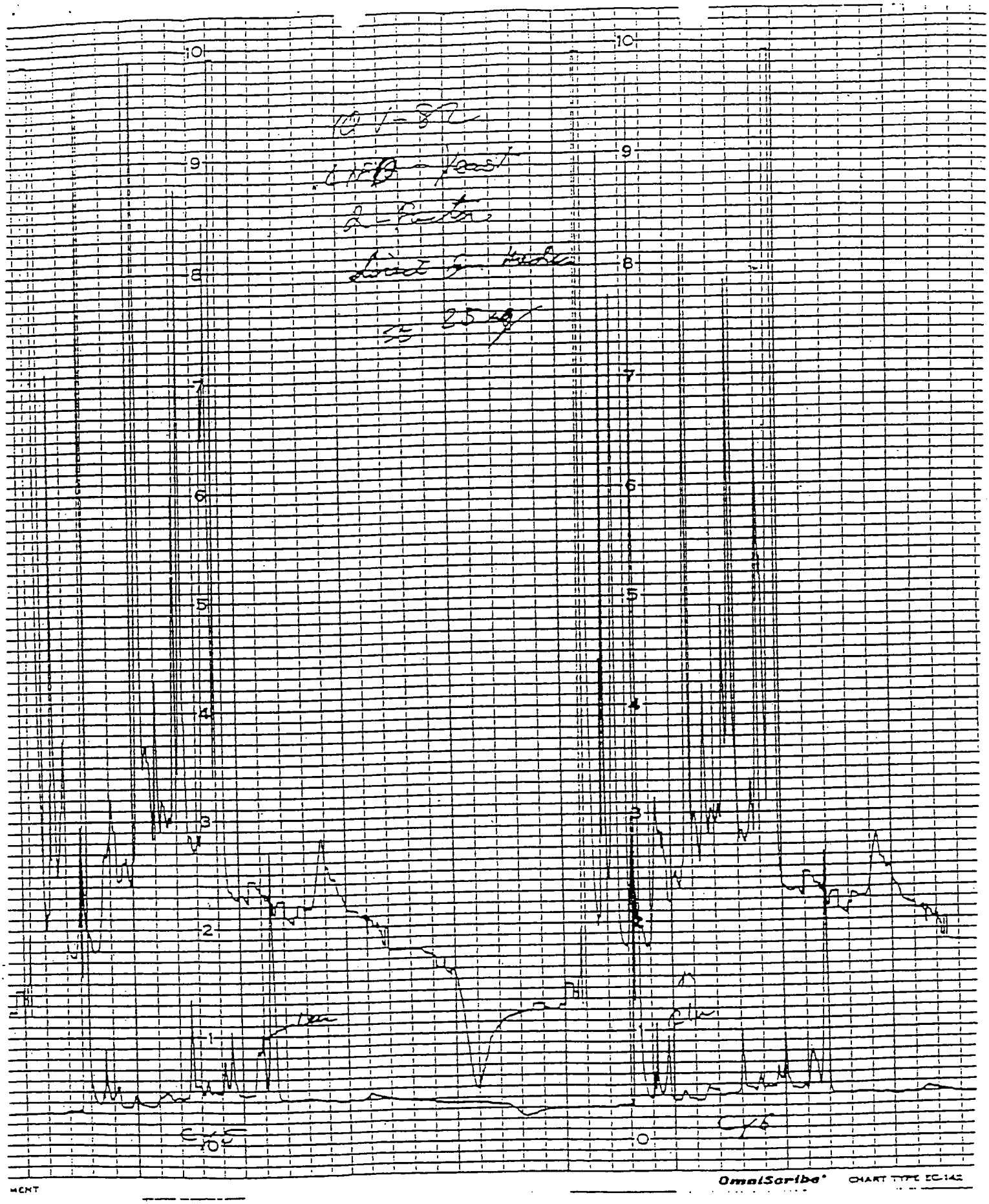
HOUSTON INSTRUMENT

000205



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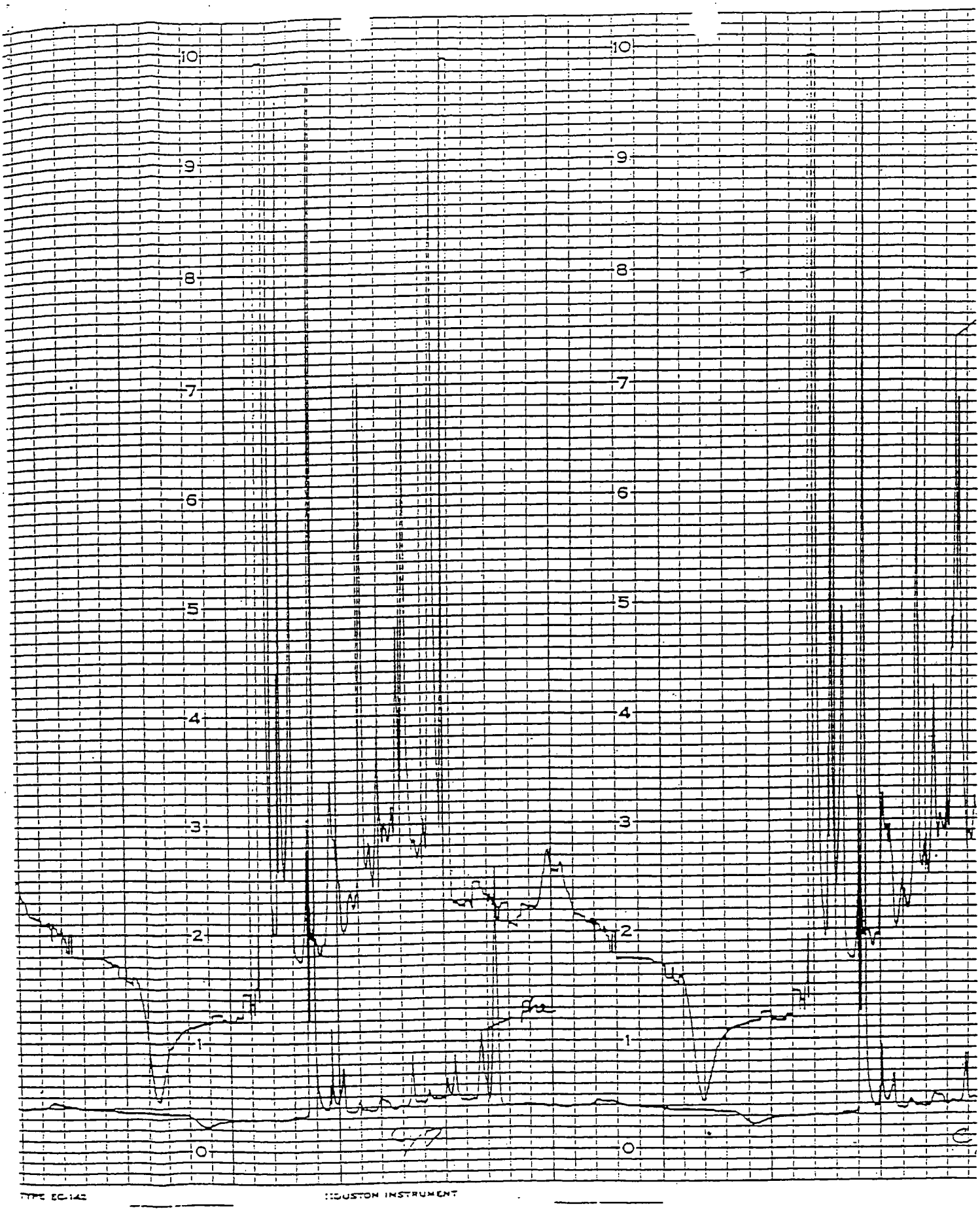

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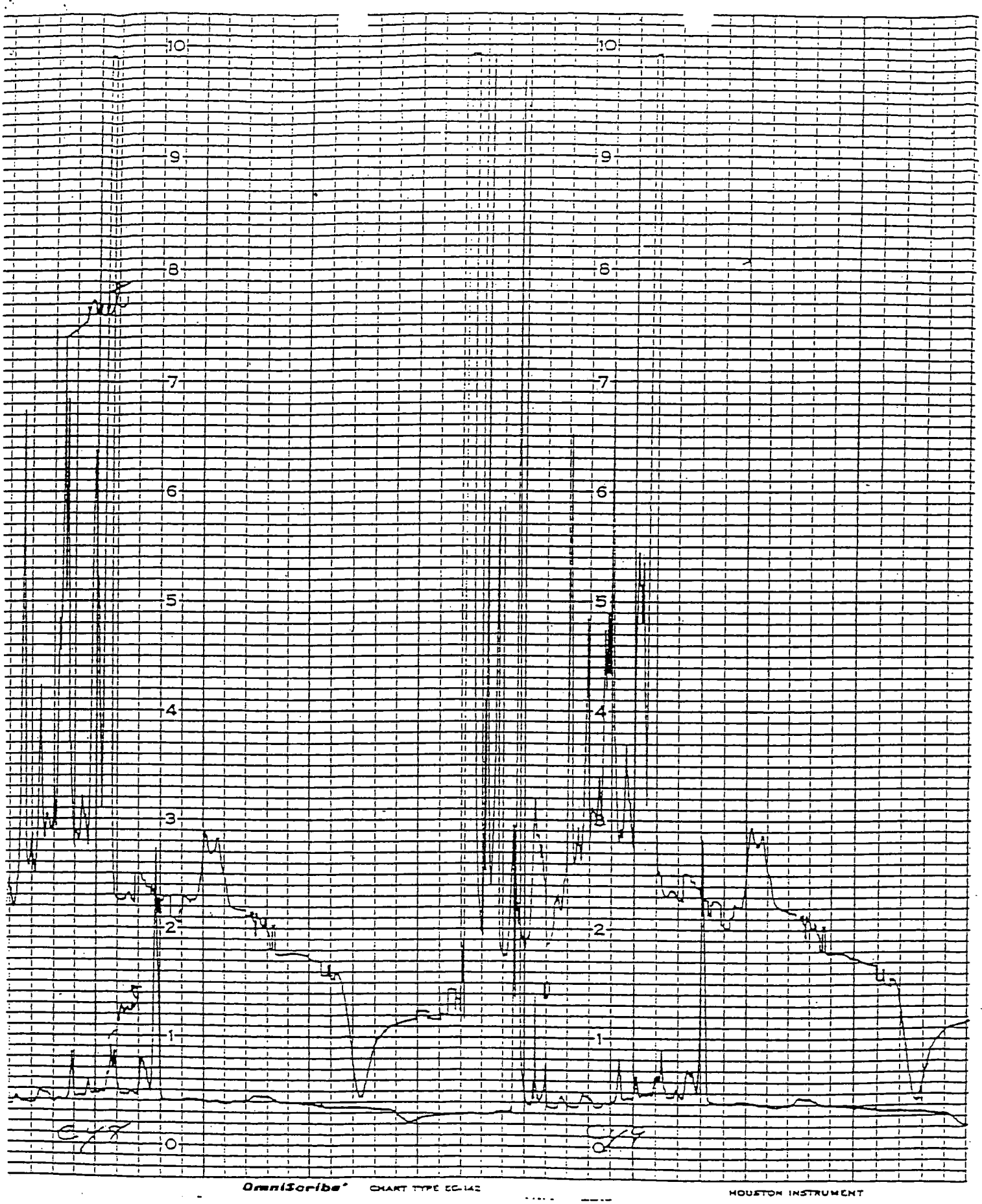
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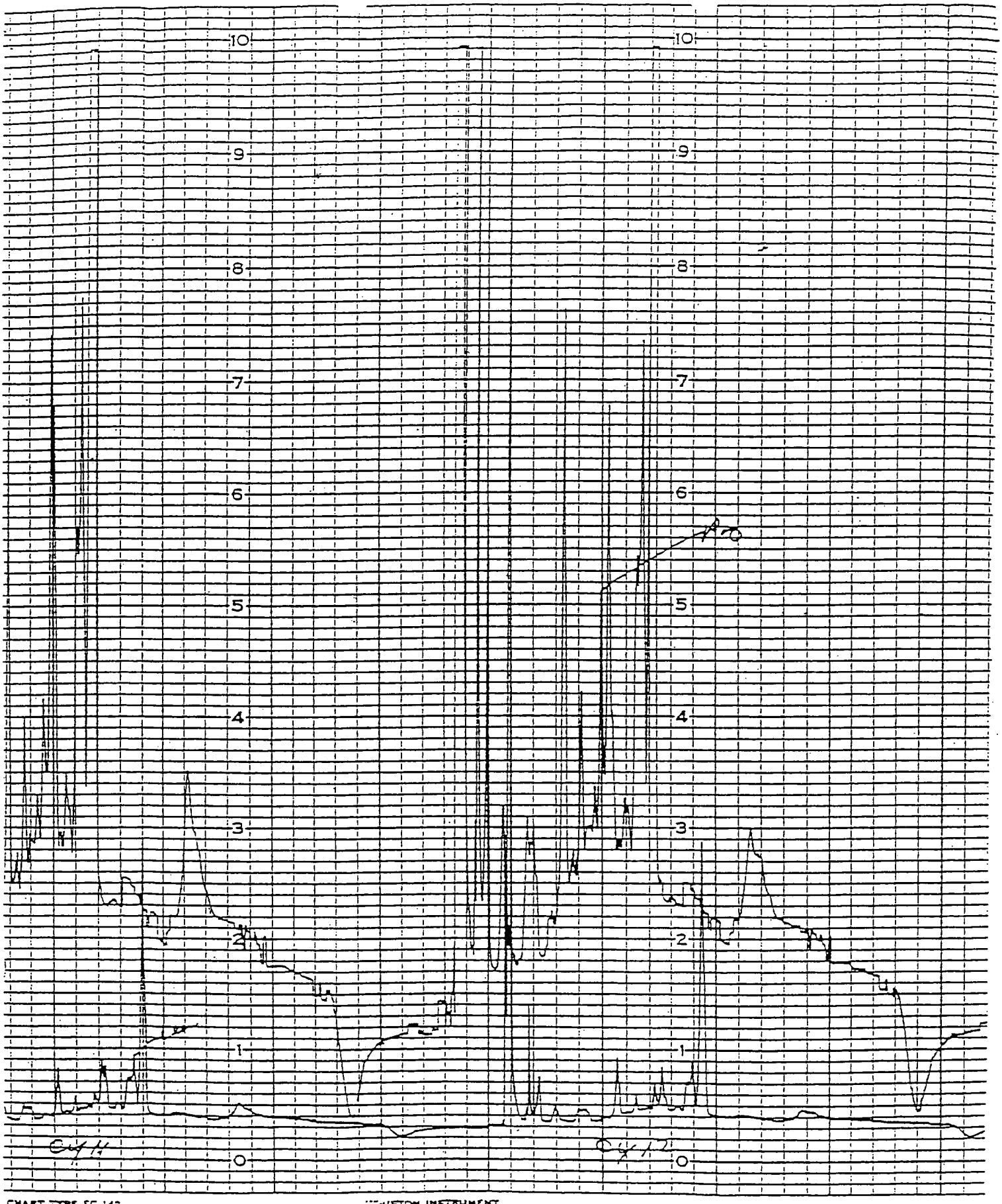
HOUSTON INSTRUMENT

000208

000209



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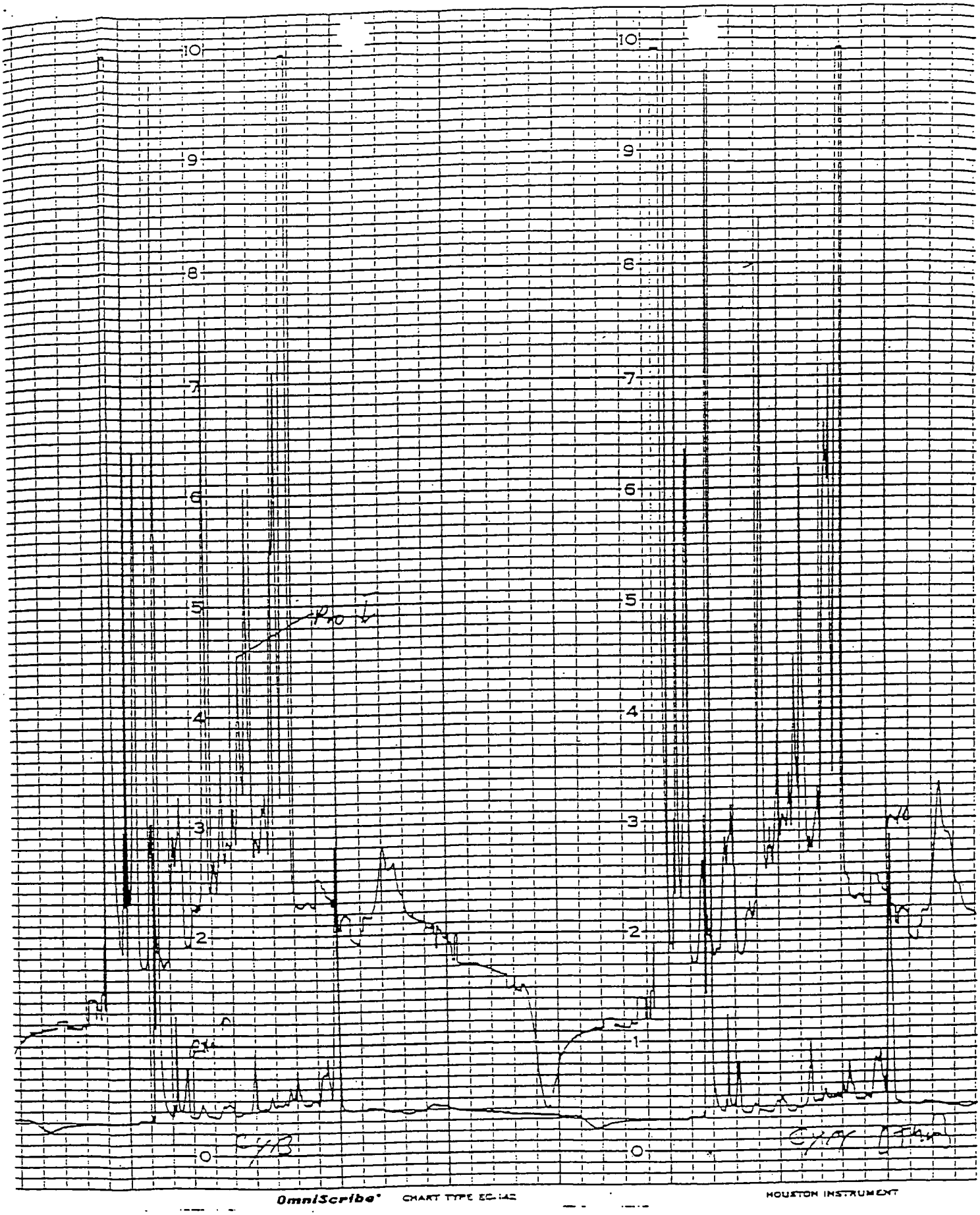
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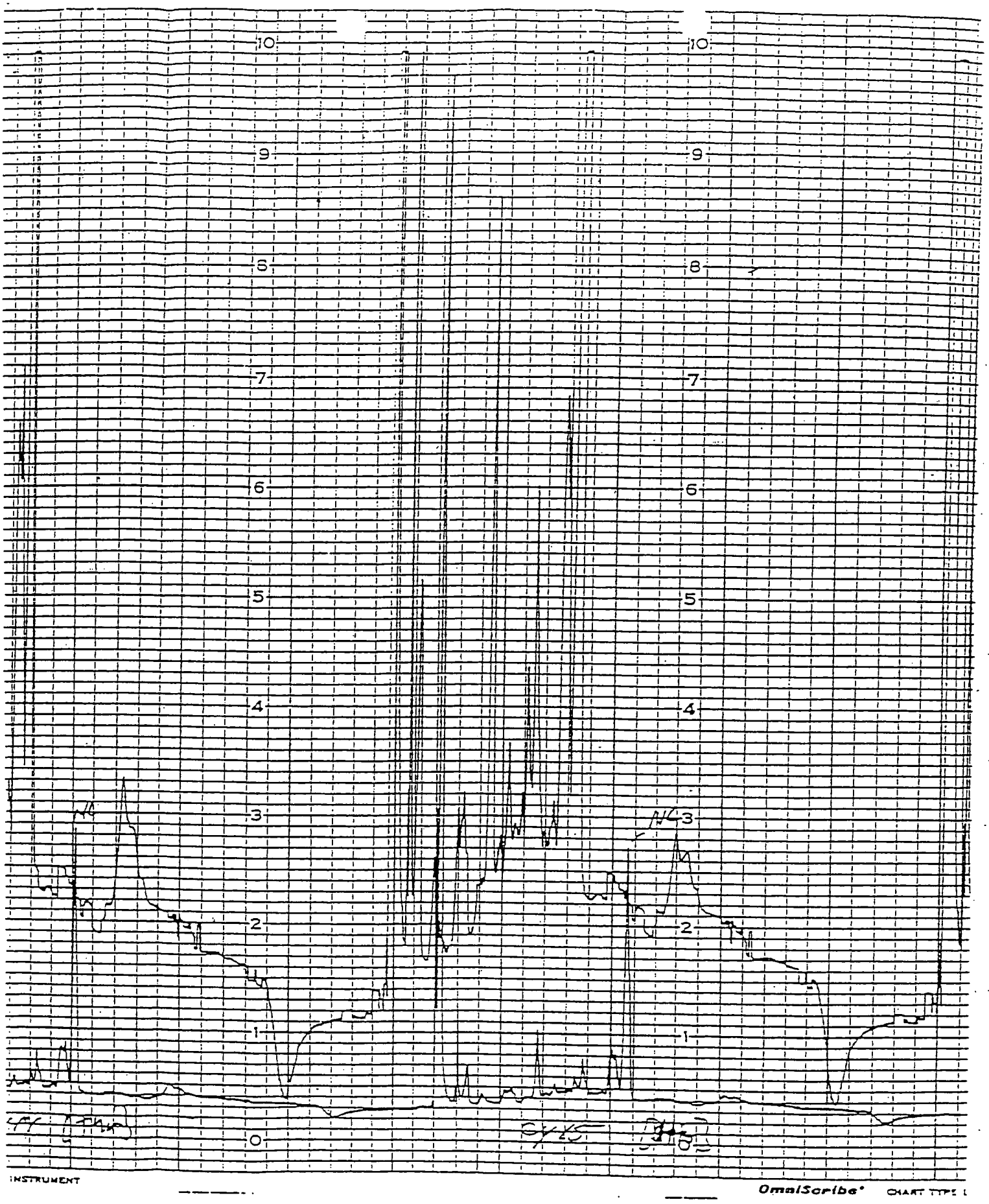
000211



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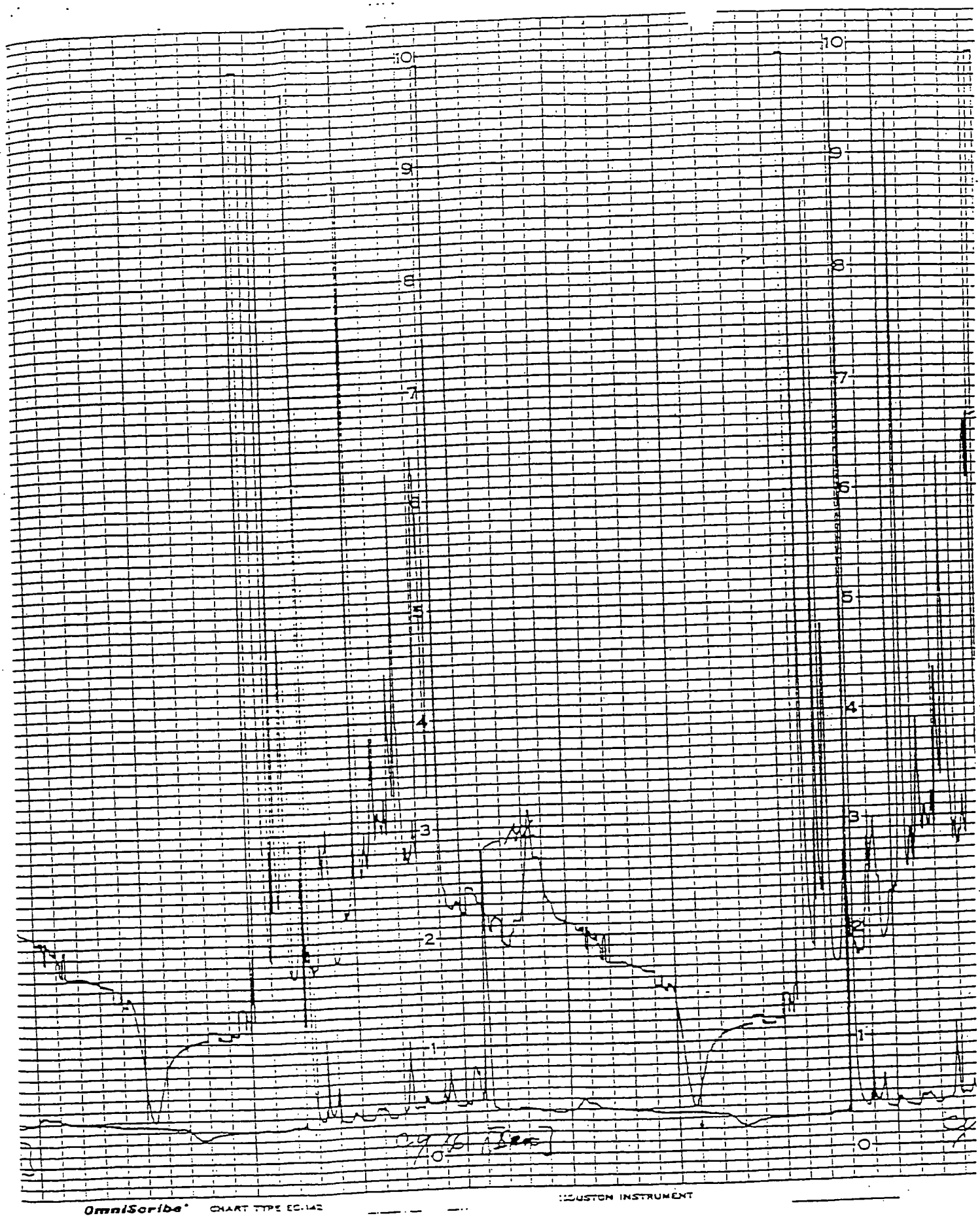
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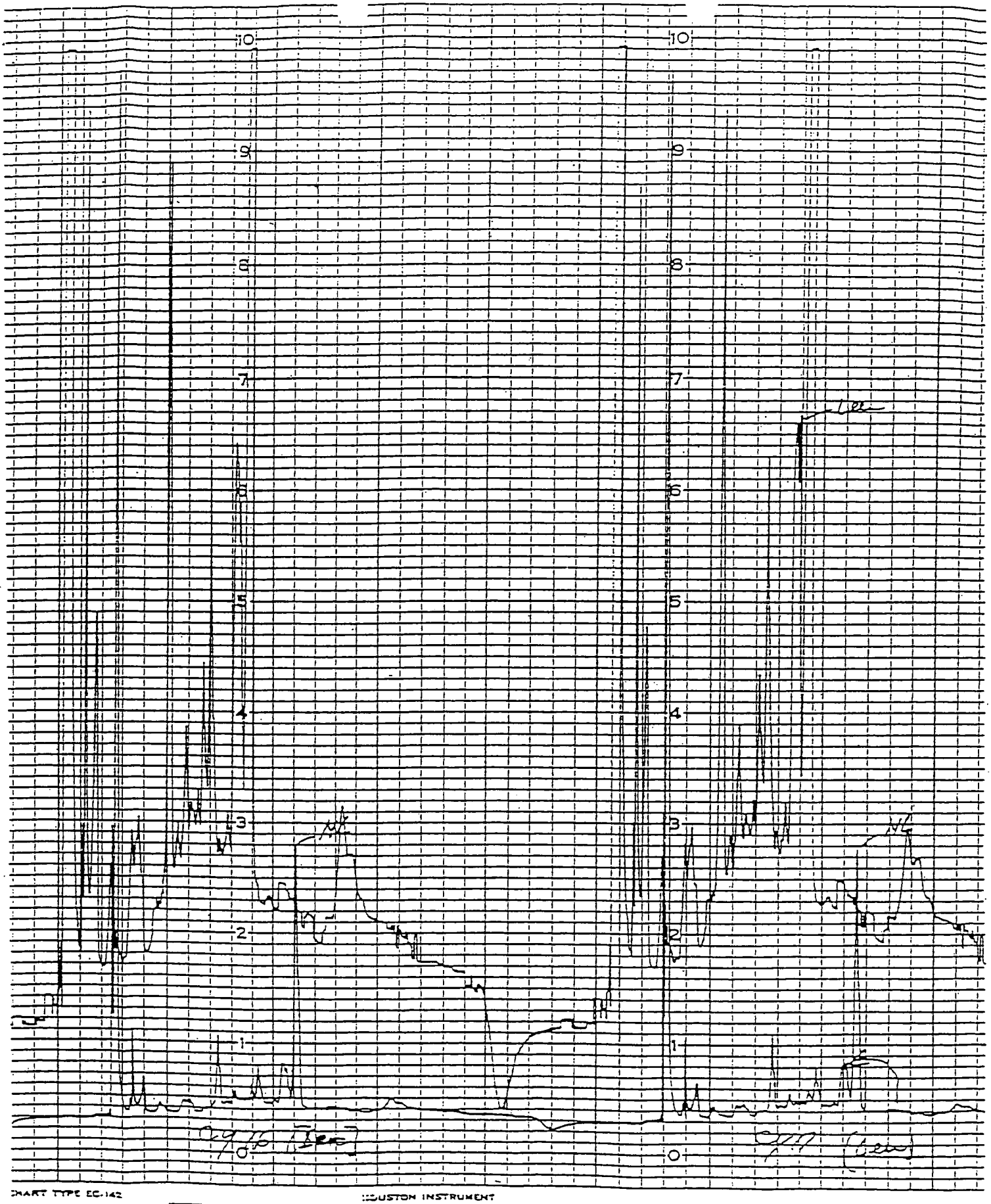

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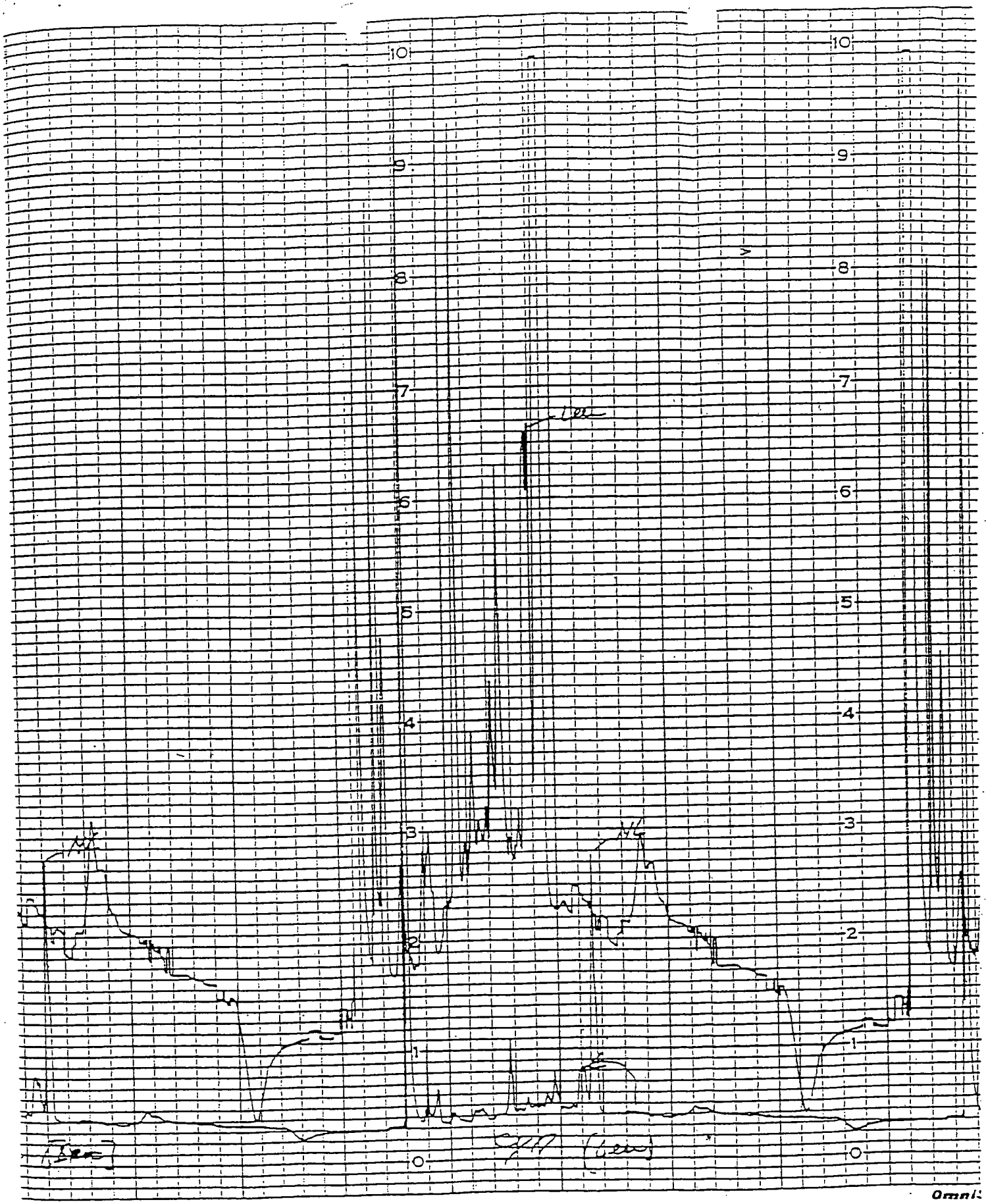

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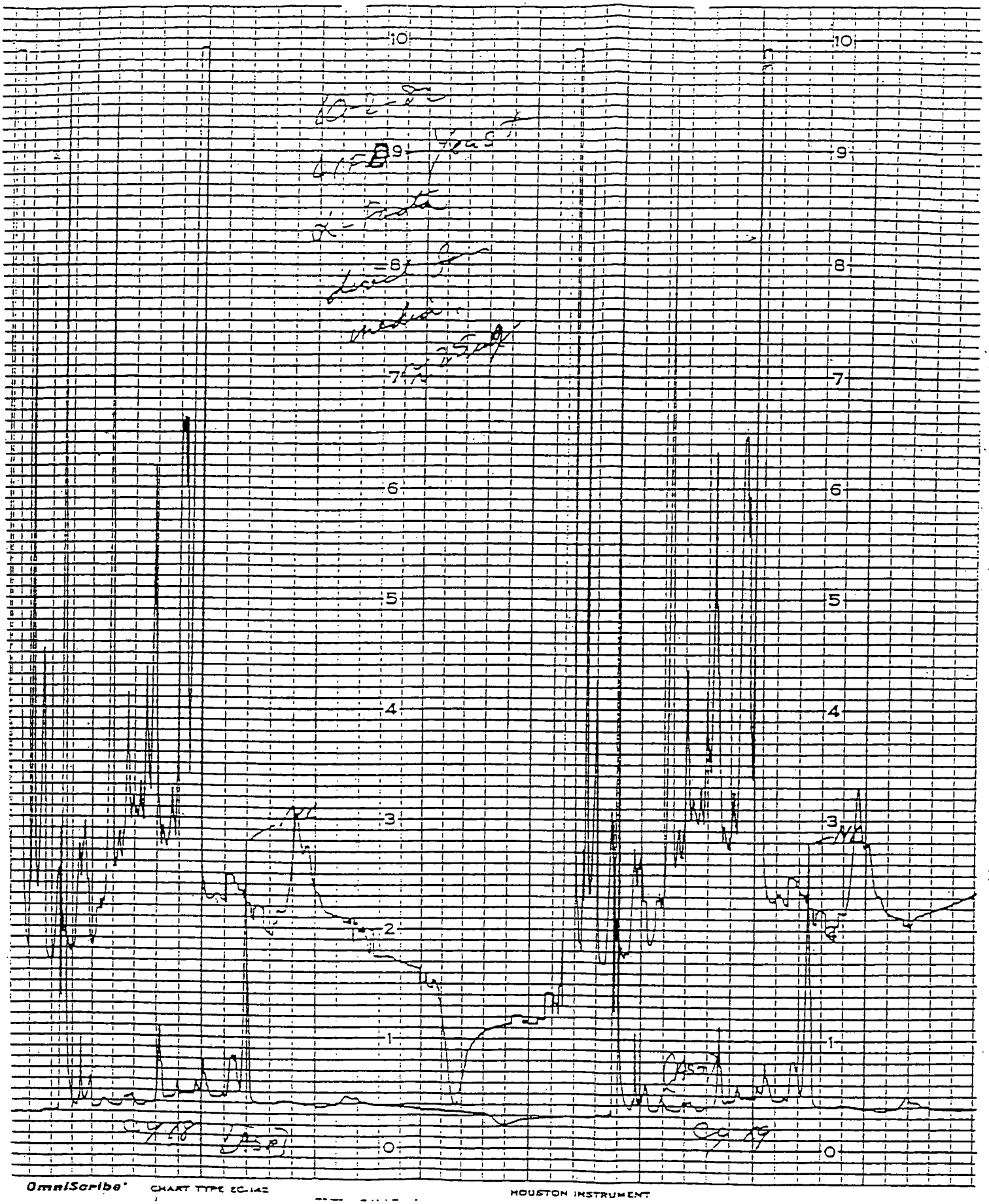
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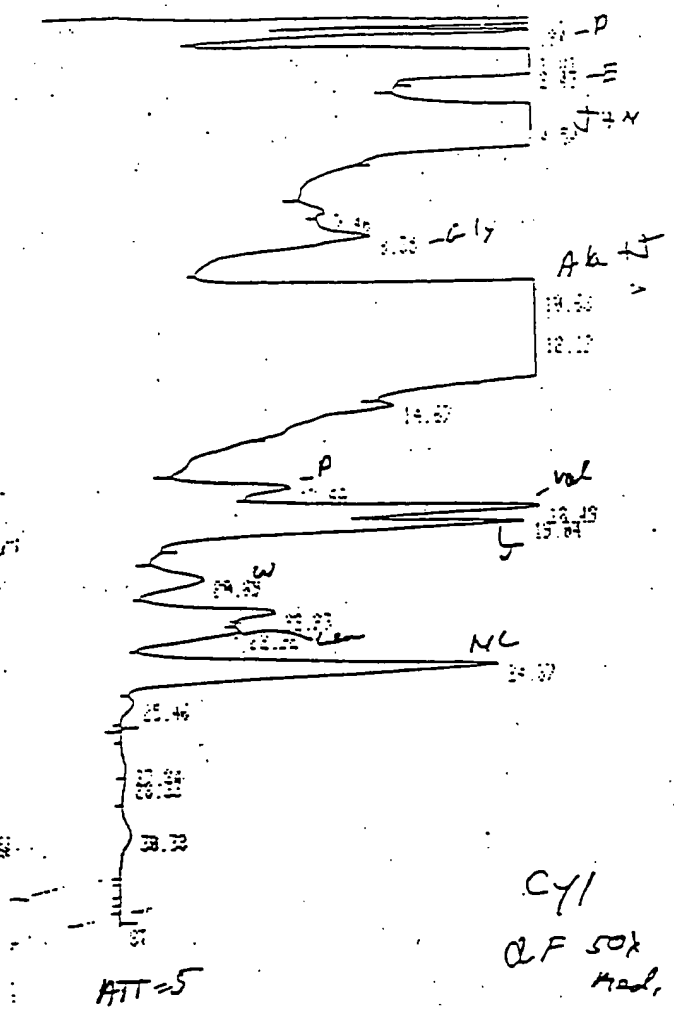
OmniScribe
CHART TYPE EC-1142
HOUSTON INSTRUMENT

C00216



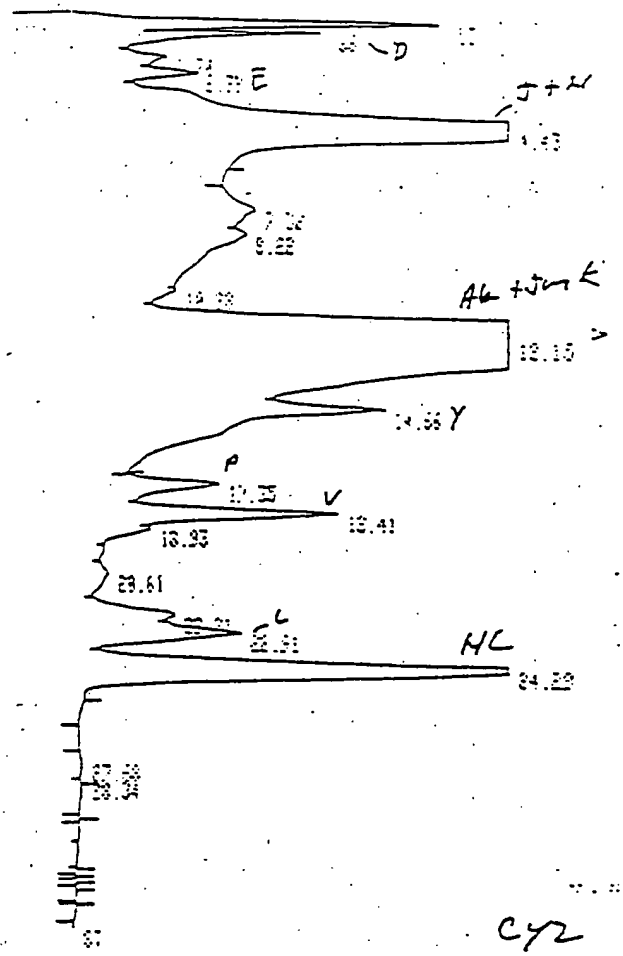
RUN # 935 OCT/01/92 14:53:43
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.53	6357500	BV	0.246	1.418
0.84	3264500	VP	0.191	0.727
1.81	1.2337E+07	PV	0.466	2.746
2.43	4357400	VB	0.352	0.970
4.54	1.6449E+08	BB	0.647	36.618
7.46	671010	BV	0.454	0.149
8.35	3012300	VP	0.726	0.671
10.63	1.5132E+08	PV	1.034	33.688
12.13	7.5634E+07	VB	0.831	16.851
14.67	353329	BB	0.315	0.079
17.50	2327000	BV	0.567	0.518
18.49	6378200	VV	0.489	1.420
19.84	4923400	VB	0.411	1.096
20.89	1248300	BP	0.620	0.278
22.23	2498300	PV	0.533	0.556
22.93	1774900	VV	0.490	0.395
24.37	7493400	VV	0.687	1.668
25.46	183870	VP	0.553	0.041
27.84	122100	VV	0.794	0.027
38.32	483040	VP	1.122	0.098

TOTAL AREA= 4.4919E+08
AMUL FACTOR= 1.0000E+00

000217



ATT=5

Cyz
LIFD-d Feat
dia. mod

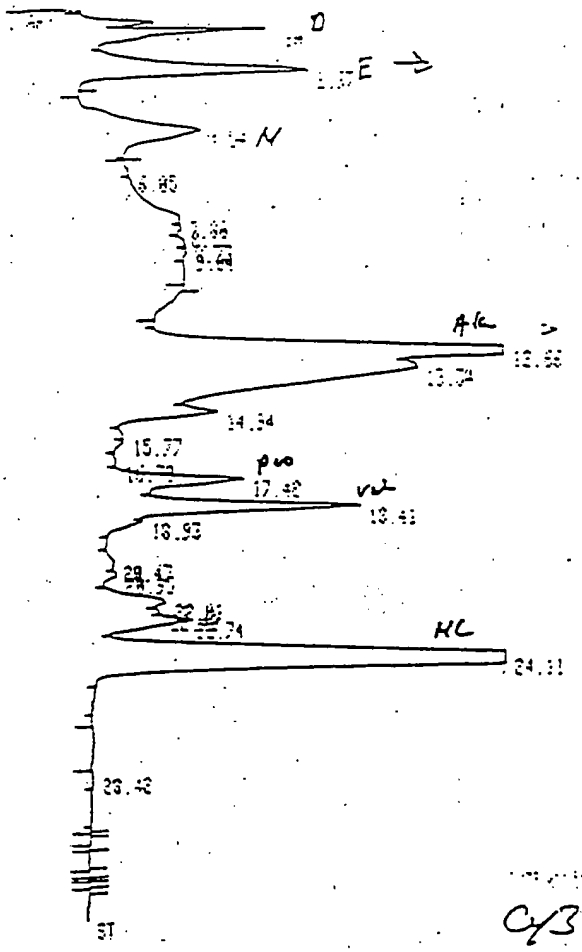
RUN # 936 OCT/01/92 16:11:44
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.53	3110200	BV	0.275	2.981
0.86	1846300	VV	0.258	1.723
1.74	706700	VV	0.437	0.659
2.38	767820	VP	0.336	0.716
4.43	1.9316E+07	PB	0.795	18.013
7.32	1514100	BV	0.932	1.412
9.22	2192100	VV	1.197	2.045
10.20	209900	VP	0.344	0.196
12.15	4.9595E+07	PV	1.333	46.265
14.66	7691900	VB	0.934	7.175
17.35	1459800	BV	0.458	1.362
18.41	3216200	VV	0.432	3.000
18.93	483710	VP	0.342	0.451
20.61	261700	PP	0.739	0.244
22.21	1423600	PV	0.497	1.328
22.91	3120100	VV	0.612	2.911
24.29	1.0289E+07	VB	0.597	9.598

TOTAL AREA= 1.0720E+08
MUL FACTOR= 1.0000E+00

000218



ATT=5

C/3

LIFD-Q-Factor
med

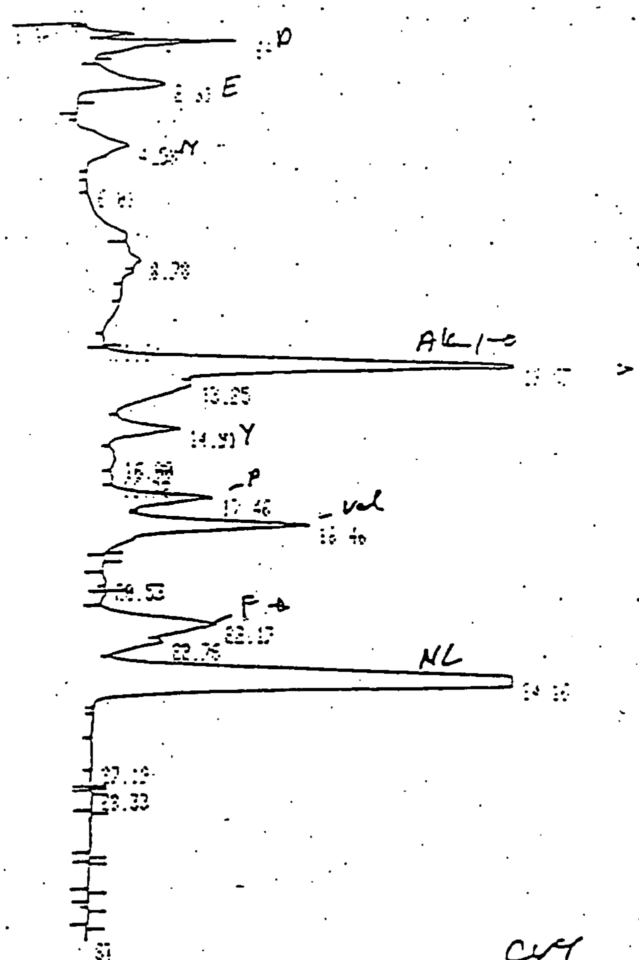
RUN # 937 OCT/01/92 17:29:46
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.50	578520	PV	0.241	1.117
0.88	1834500	VV	0.384	3.593
2.37 E	3724900	VB	0.498	7.285
4.54	2328800	PV	0.744	4.561
7.38	294700	PV	0.387	0.577
8.13	240750	VV	0.398	0.472
8.73	164038	VV	0.400	0.321
9.84	101850	VB	0.362	0.199
12.66	8472300	VV	0.541	16.593
13.34	9265500	VV	1.082	18.147
14.94	1264300	VP	0.411	2.476
17.42	1982100	VV	0.463	3.882
18.41	3473000	VV	0.428	6.802
18.93	362570	VV	0.325	0.710
20.47	155730	PV	0.468	0.305
20.95	154200	VP	0.335	0.302
22.81	1033800	PV	0.496	2.023
22.40	422140	VV	0.216	0.827
22.74	1443900	VV	0.513	2.848
24.11	1.3765E+07	VV	0.599	26.959

TOTAL AREA= 5.1059E+07
MUL FACTOR= 1.0000E+00

000219



ATT 5

CYT
LIFD-α-Factor
rad.

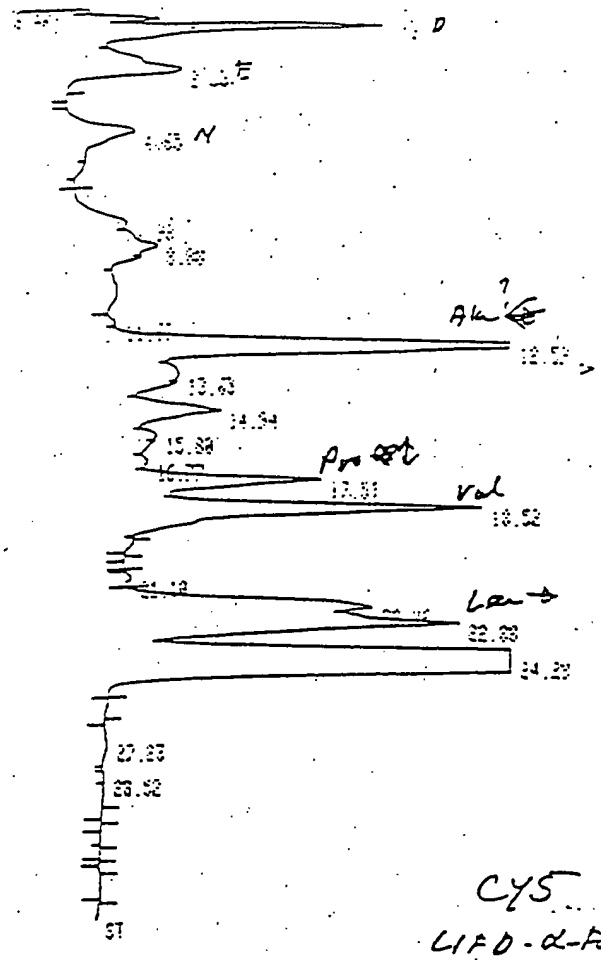
RUN # 938 OCT/01/82 18:47:48
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.40	263560	PY	0.177	0.717
0.69	1153000	YB	0.245	3.152
2.31	1287700	BB	0.498	3.585
4.58	1182200	PY	0.729	3.218
8.78	335460	BY	0.522	0.913
12.57	7266500	BY	0.456	19.777
13.25	2112900	YY	0.752	5.751
14.91	1122100	YY	0.465	3.054
16.00	231460	YY	0.686	0.630
16.72	100430	YY	0.409	0.273
17.46	1553600	YY	0.445	4.242
18.46	3182000	YB	0.476	8.663
22.17	2558200	BY	0.678	6.964
22.76	897978	YY	0.420	2.444
24.16	1.3484E+07	YY	0.624	36.699

TOTAL AREA= 3.6743E+07
MUL FACTOR= 1.0000E+00

000220



CYS
CIFO - α-Fmoc
mod.

ATT=4

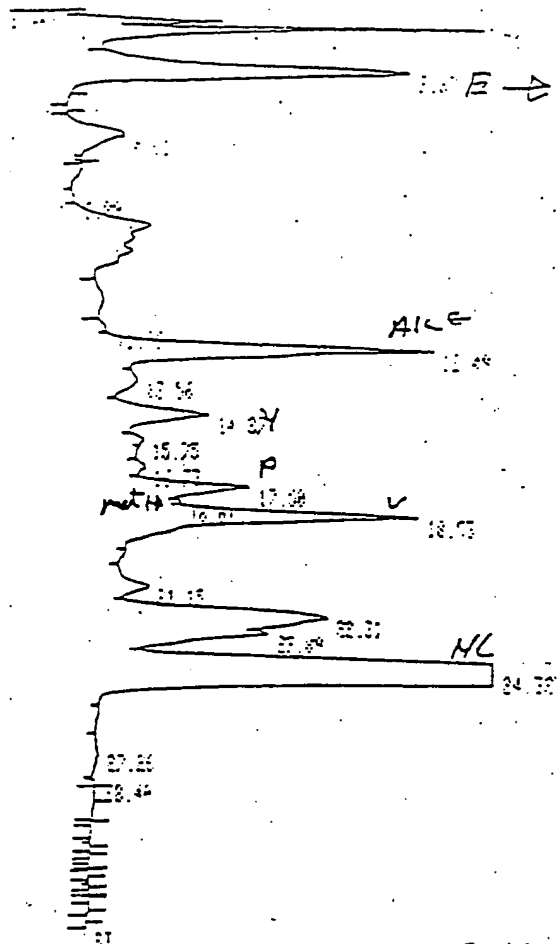
RUN # 939 OCT/01/92 20:05:50
ID 891

AREA%

RT		AREA	TYPE	AR/HT	AREA%
8.40		238960	BV	0.283	0.676
8.74		1783780	VV	0.355	4.875
9.33		1353200	VB	0.748	3.827
4.65		858350	BV	0.768	2.405
7.98		276390	PV	0.454	0.782
8.88		685180	VV	0.733	1.938
12.59	Ala	4060880	VV	0.460	11.486
13.63		672250	VV	0.681	1.901
14.34		996490	VV	0.587	2.819
15.89		225950	VV	0.377	0.639
16.77		177450	VV	0.439	0.502
17.51		1476180	VV	0.461	4.175
18.52		2963180	VV	0.512	8.380
22.26		2253680	BV	0.558	6.378
22.93		3611380	VV	0.647	10.214
24.28		1.3779E+07	VB	0.639	38.974

TOTAL AREA= 3.5355E+07
MUL FACTOR= 1.0000E+00

000221



ATT-4

CY6
LIFD-α factor
med.

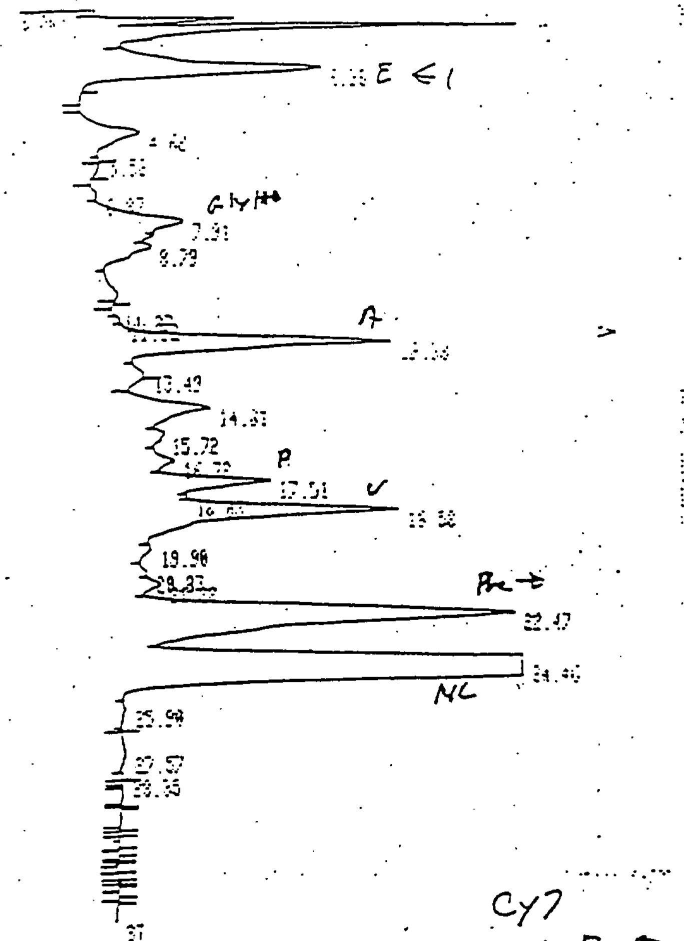
RUN # 940 OCT/01/92 21:23:51
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.41	332800	PV	0.161	0.985
0.71	949190	VB	0.155	2.810
2.37	2783400	BB	0.511	8.240
4.61	543720	BV	0.642	1.610
12.49	2663200	VV	0.476	7.298
13.56	569170	VV	0.606	1.685
14.67	1167300	VV	0.622	3.456
15.75	327640	VV	0.421	0.970
16.73	469640	VV	0.579	1.390
17.50	1332500	VV	0.522	3.945
18.01	304470	VV	0.211	0.901
18.53	3272800	VV	0.686	9.688
21.15	723460	VV	0.780	2.142
22.31	3868300	VV	0.784	9.883
22.89	1333100	VV	0.458	3.946
24.32	1.3936E+07	VV	0.656	41.253

TOTAL AREA= 3.3782E+07
MUL FACTOR= 1.0000E+00

000222



ATT-4

CY7
LIFD d-Factor

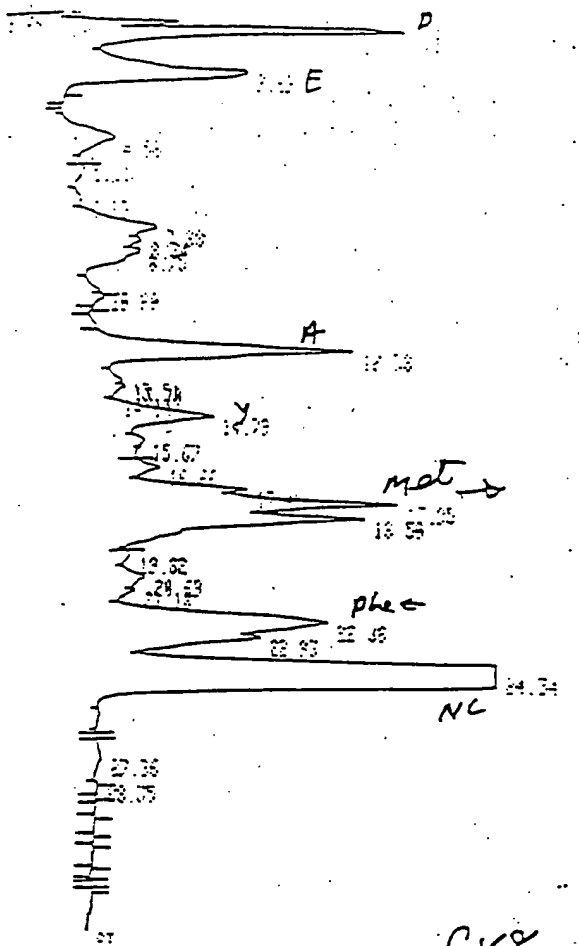
RUN # 941 OCT/01/92 22:41:52
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.44	373550	PV	0.173	1.147
0.73	1249900	VB	0.181	3.839
2.33	1904600	BB	0.521	5.849
4.62	484430	PV	0.575	1.489
7.91	898620	VV	0.621	2.760
8.79	448160	VB	0.524	1.376
12.33	1872900	VP	0.430	5.770
14.81	981630	VV	0.647	2.708
15.72	373130	VV	0.683	1.146
16.72	510190	VV	0.662	1.567
17.51	1201600	VV	0.587	3.690
18.03	188580	VV	0.186	0.579
18.53	2695300	VV	0.681	8.277
19.90	262550	VV	0.614	0.806
20.83	150790	VV	0.418	0.463
21.28	336650	VV	0.570	1.034
22.47	5237700	VV	0.812	16.085
24.46	1.3486E+07	VV	0.648	41.416

TOTAL AREA= 3.2563E+07
MUL FACTOR= 1.0000E+00

000223



ATT=4

Cy8
LIFD-α-Fmoc
mal

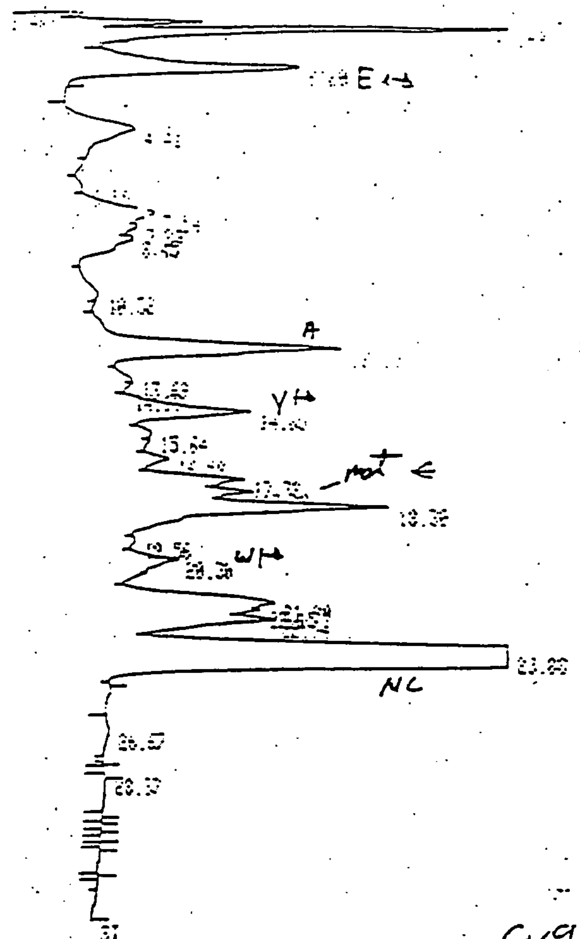
RUN # 942 OCT/01/92 23:59:54
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.37	353910	BV	0.216	1.138
0.71	1854000	VV	0.341	5.964
2.29	1986200	VB	0.659	6.282
4.58	427100	PV	0.689	1.351
7.09	742400	VV	0.578	2.349
8.34	374430	VV	0.375	1.184
9.75	560620	VP	0.575	1.773
12.38	1996100	PV	0.452	6.829
14.79	773600	VV	0.495	2.467
16.66	151840	BV	0.346	0.478
17.45	574600	VV	0.295	1.816
17.95	2051900	VV	0.465	6.498
18.50	1929200	VP	0.493	6.102
20.69	188800	PV	0.456	0.595
22.33	2544700	VV	0.788	8.049
22.93	1094700	VV	0.432	3.463
24.34	1.4091E+07	VP	0.637	44.578

TOTAL AREA= 3.1615E+07
MUL FACTOR= 1.0000E+00

000224



ATT=4

C49
LIFD-02-Fat
pad.

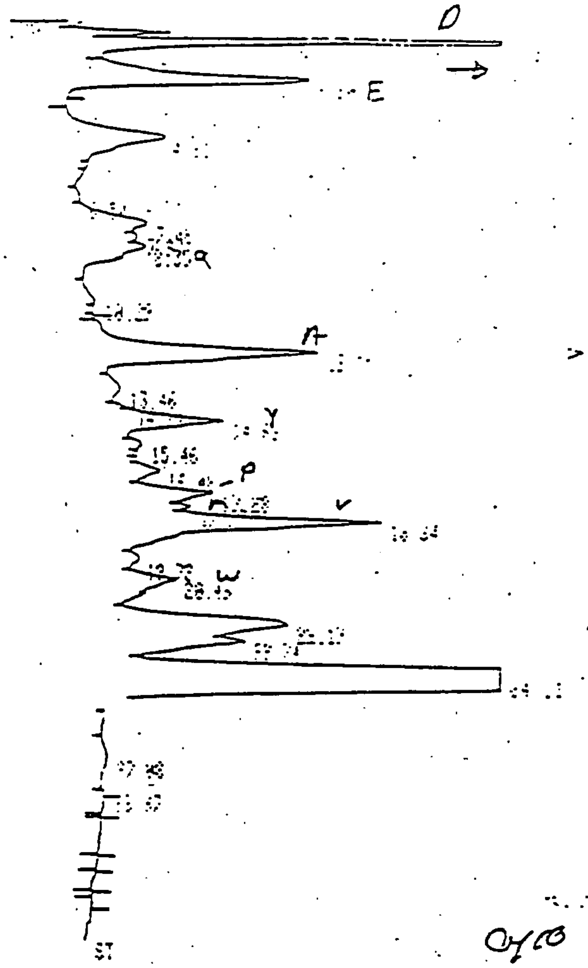
RUN # 343 OCT/02/82 01:17:55
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.33	361140	PV	0.184	0.929
0.69 D	1634900	VB	0.175	4.476
2.20	1719500	BB	0.463	4.708
4.41	1025000	PV	0.905	2.811
7.54	906300	VV	0.661	2.431
7.99	414690	VV	0.393	1.135
8.45	605120	VV	0.622	1.657
10.53	230060	VV	0.712	0.638
12.51	2512500	VV	0.582	6.873
13.69	372130	VV	0.400	1.019
14.85	1675600	VV	0.513	4.587
15.64	472770	VV	0.450	1.294
16.58	733000	VV	0.564	2.007
17.32	1217400	VV	0.477	3.333
17.78	949700	VV	0.354	2.600
18.30	2816600	VV	0.581	7.711
19.56	310770	VV	0.491	0.851
20.30	759970	VV	0.581	2.021
21.90	1533700	VV	0.529	4.199
22.15	687460	VV	0.253	1.882
22.54	1373200	VV	0.482	3.759
23.88	1.4214E+07	VP	0.618	38.913

TOTAL AREA= 3.5527E+07
MUL FACTOR= 1.0000E+00

000225



ATT=4

Cy CO
LIFD a-Fecta
med.

RUN # 944
ID 091

UCT/00/92 02:35:56

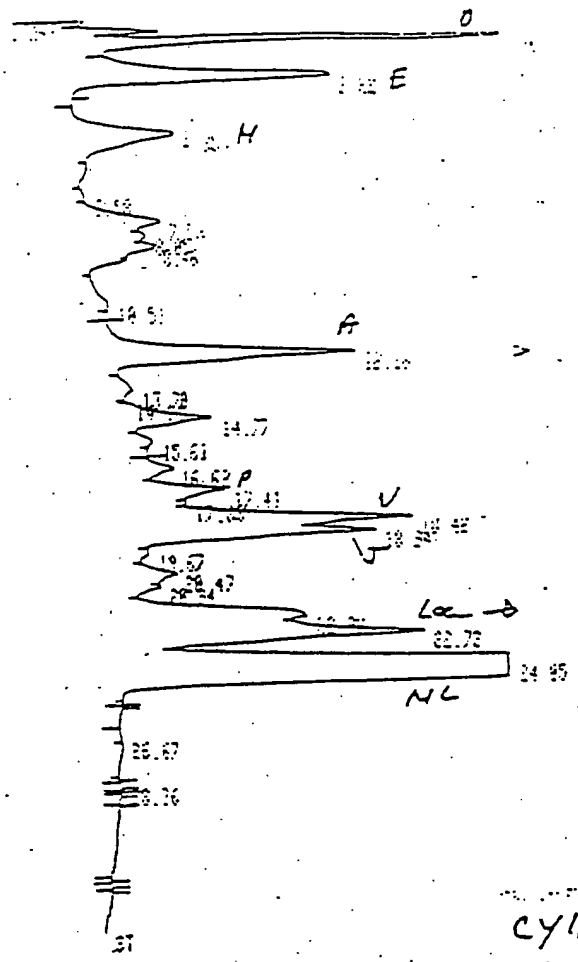
AREA%

RT		AREA	TYPE	AR/HT	AREA%
0.42		214900	PP	0.161	0.612
0.73	D	2509700	PB	0.134	7.146 /Hy
2.20		1707700	BB	0.449	4.862
4.31		1211400	PV	0.755	3.449
7.40		711490	VV	0.616	2.026
7.97		331050	VV	0.345	0.943
8.35		478040	VV	0.443	1.361
12.15		1943700	BV	0.522	5.534
13.46		314660	VV	0.796	0.896
14.14		186040	VV	0.289	0.382
14.61		1124500	VV	0.530	3.202
15.46		321320	VV	0.431	0.915
16.48		632070	VV	0.683	1.800
17.23		1039300	VV	0.537	2.959
17.79		413800	VV	0.265	1.178
18.34		3013000	VV	0.637	8.579
19.72		398450	VV	0.568	1.135
20.49		750650	VV	0.580	2.137
22.13		2329600	VV	0.733	6.633
22.74		1193200	VV	0.489	3.397
24.11		1.4270E+07	VV	0.625	40.628
27.86		188618	VP	0.959	0.389

TOTAL AREA=3.5123E+07
MUL FACTOR=1.0000E+00

000226

A472



ATT-4

CYII

LIFD-α-Factor
mod.

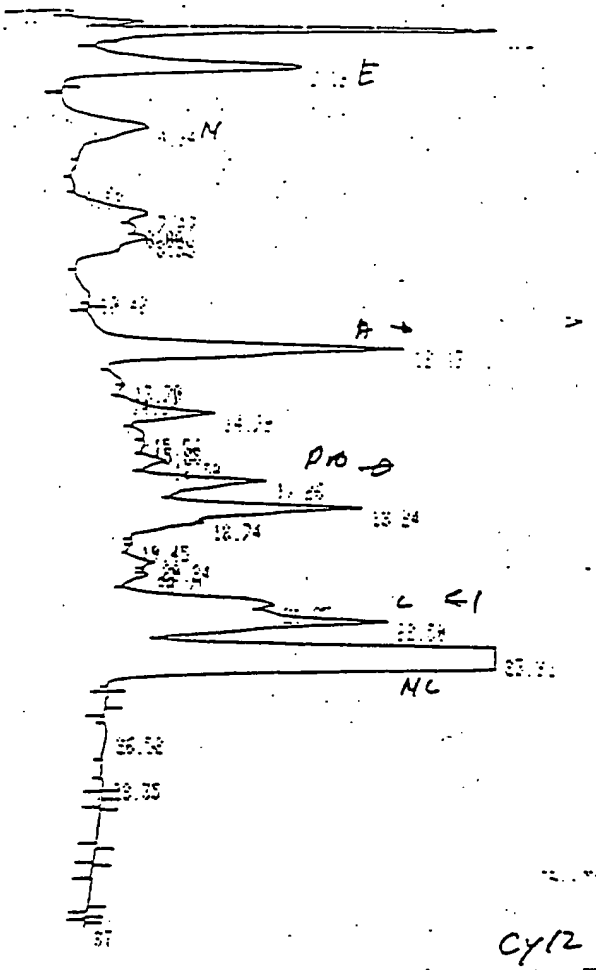
RUN # 945 OCT/02/92 03:53:58
ID 391

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.45	213470	PV	0.181	0.648
0.76	1965500	VB	0.155	5.831
2.22	1940500	BB	0.475	5.757
4.35	1186500	BV	0.699	3.520
7.53	769370	VV	0.681	2.282
8.85	378290	VV	0.371	1.122
9.46	529390	VV	0.458	1.570
12.38	1887500	PV	0.468	5.599
14.77	575240	VV	0.419	1.706
16.62	161290	PV	0.357	0.479
17.41	580480	VV	0.436	1.722
17.88	166410	VV	0.217	0.494
18.42	2073100	VV	0.459	6.150
18.94	1561100	VV	0.398	4.631
20.47	389310	VV	0.458	0.918
20.94	132910	VV	0.385	0.394
22.89	1533900	VV	0.523	4.550
22.72	3069600	VV	0.623	9.106
24.85	1.4672E+07	VP	0.617	43.522

TOTAL AREA= 3.3711E+07
MUL FACTOR= 1.0000E+00

000227



ATT=4

Cyl2
LIFD-a-Factor
mod.

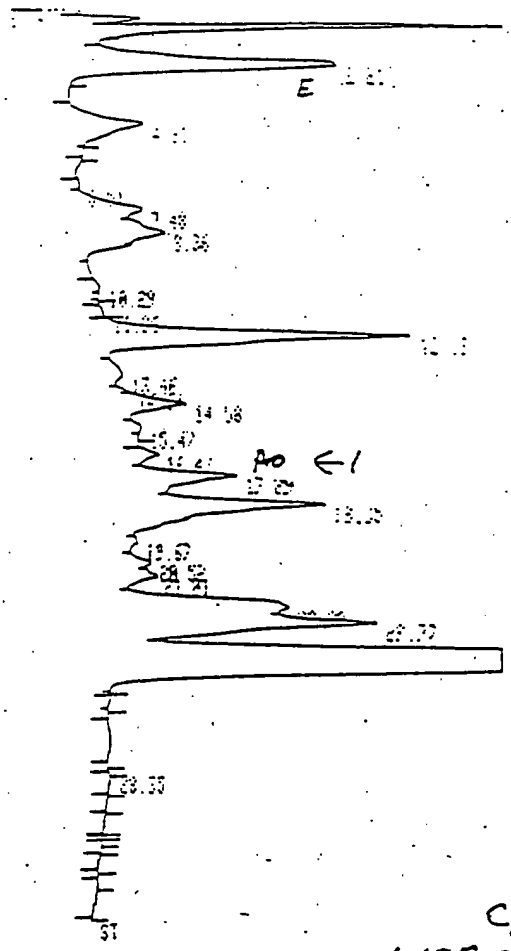
RUN # 346 07/02/92 05:11:59
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.42	216658	PV	0.263	0.586
0.72	1905100	VB	0.194	5.152
2.13	1807300	BB	0.484	4.887
4.34	1139900	PV	0.824	3.083
7.47	774550	VV	0.612	2.095
8.00	376950	VV	0.368	1.019
8.35	775250	VP	0.641	2.097
12.43	2675400	PV	0.511	7.235
13.70	257720	VV	0.473	0.697
14.79	1161800	VV	0.578	3.142
15.56	362140	VV	0.451	1.033
15.88	369860	VV	0.439	0.998
16.58	638140	VV	0.549	1.726
17.26 Pro	1645800	VV	0.582	4.451
18.24	2173700	VV	0.495	5.878
18.74	731888	VV	0.416	1.979
19.45	125800	VV	0.228	0.328
20.24	379300	VV	0.434	1.026
20.71	304200	VV	0.394	0.823
21.85	1541200	VV	0.548	4.168
22.58	3049600	VV	0.646	8.247
23.81	1.4548E+07	VP	0.617	39.341

TOTAL AREA= 3.6978E+07
MUL FACTOR= 1.0000E+00

000228



ATT=4

CYB
LIFD-2-Feeder
mod.

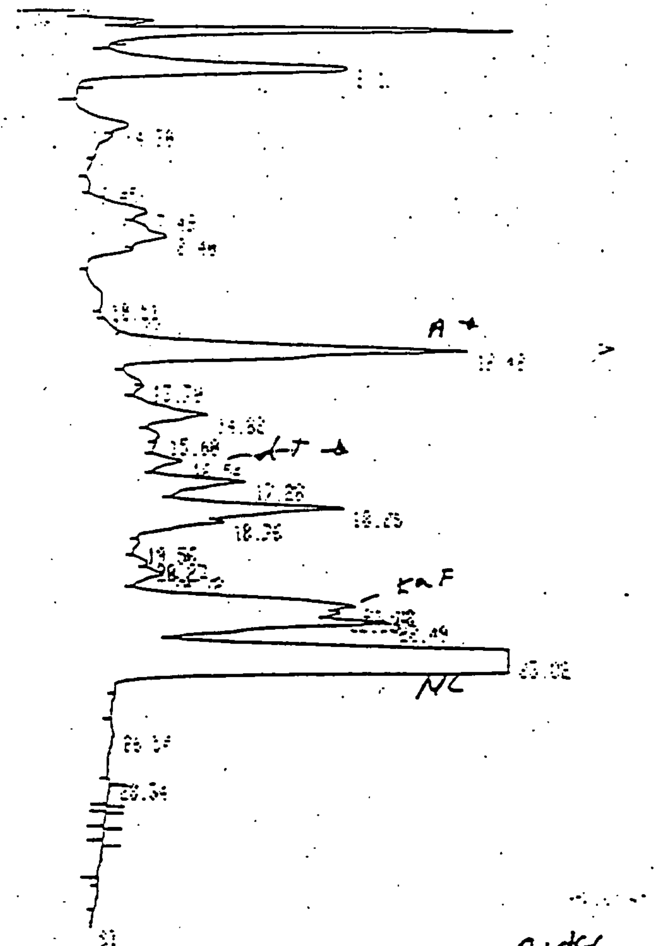
RUN # 247 OCT-12/92 06:30:01
ID 891

AREA%

-RT	AREA	TYPE	AR/HT	AREA%
0.40	194338	PV	0.197	0.635
0.69	1616488	VB	0.189	5.278
2.21 E	2223788	BB	0.519	7.262 1/49
4.31	722518	PV	0.629	2.359
7.48	606668	VV	0.571	1.981
8.36	962618	VV	0.698	3.143
12.12	2278888	BP	0.451	7.439
14.58	468418	VV	0.425	1.538
16.47 dt	132538	BV	0.357	0.433
17.29	918278	VV	0.523	2.999
18.35	1593488	VV	0.498	5.203
20.52	155338	VV	0.336	0.507
21.81	281368	VV	0.482	0.919
22.28	1683888	VV	0.581	5.499
22.77	2545588	VV	0.585	8.312
24.13	1.4248E+87	VP	0.625	46.582

TOTAL AREA= 3.0623E+07
MUL FACTOR= 1.0000E+00

000229



ATT=4

CYY
LIFD-α-Factor
mod.

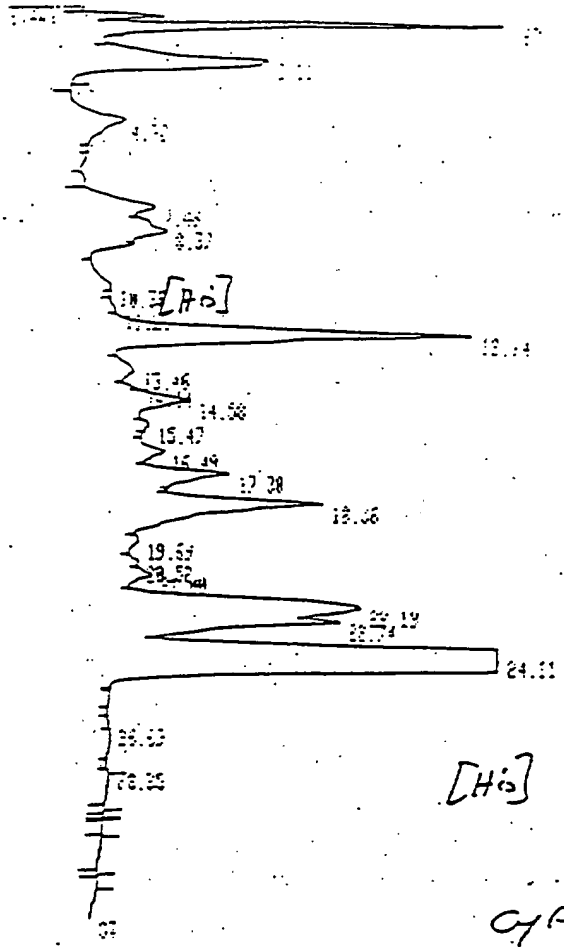
RUN # 948   OCT/02/92 07:48:03
ID 031

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.41	223830	PV	0.295	0.554
0.71	1766000	VB	0.215	4.389
2.17	2115200	BB	0.584	5.256
4.30	476120	PV	0.544	1.183
7.43	728700	VV	0.653	1.811
8.40	1079400	VV	0.762	2.682
10.51	297960	VV	0.952	0.740
12.43	3549500	VV	0.571	8.821
13.70	424010	VV	0.464	1.054
14.82	1436300	VV	0.749	3.569
15.60	522550	VV	0.455	1.299
16.54 α-T	870900	VV	0.587	2.164/.4
17.28	1563100	VV	0.629	3.884
18.26	2110200	VV	0.518	5.245
18.76	931900	VV	0.445	2.316
19.56	386030	VV	0.524	0.959
20.27	368310	VV	0.433	0.915
20.72	656030	VV	0.595	1.630
21.83	2201100	VV	0.524	5.470
22.13	985910	VV	0.249	2.450
22.43	2360600	VV	0.499	5.866
23.82	1.4999E+07	VV	0.638	37.272
26.54	189020	VP	1.234	0.470

TOTAL AREA= 4.0241E+07
MUL FACTOR= 1.0000E+00

000230



ATT=4

GB
LIFO-d factor
med.

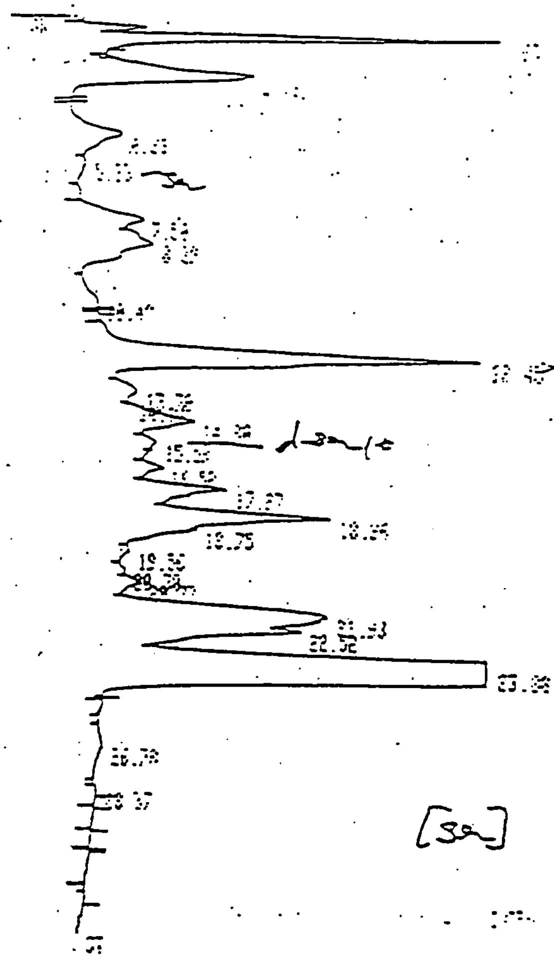
RUN # 949 OCT/02/92 09:06:05
ID 891

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.38	250420	BV	0.193	0.661
0.67	1504700	VB	0.183	3.971
2.11	1642200	BB	0.543	4.334
4.32	634870	PV	0.736	1.674
7.48	696860	BV	0.583	1.845
8.37	1001700	VV	0.726	2.644
10.38	249530	VV	0.638	0.659
11.25	222540	VV	0.486	0.587
12.14	3482300	VV	0.534	9.188
13.46	589870	VV	0.882	1.555
14.14	194610	VV	0.252	0.514
14.58	1148900	VV	0.678	3.011
15.47	463230	VV	0.485	1.223
16.49	921790	VV	0.762	2.433
17.38	1368700	VV	0.593	3.591
18.36	2855500	VV	0.739	7.537
19.69	426960	VV	0.689	1.127
20.52	318700	VV	0.447	0.838
21.88	551710	VV	0.686	1.456
22.13	3347000	VV	0.752	8.834
22.74	1892700	VV	0.465	4.996
24.11	1.4227E+07	VV	0.648	37.551

TOTAL AREA= 3.7888E+07
MUL FACTOR= 1.0000E+00

000231



[3a]

ATT=4

UFD a-factor
Cy 15
red.

RUN # 950
ID 391

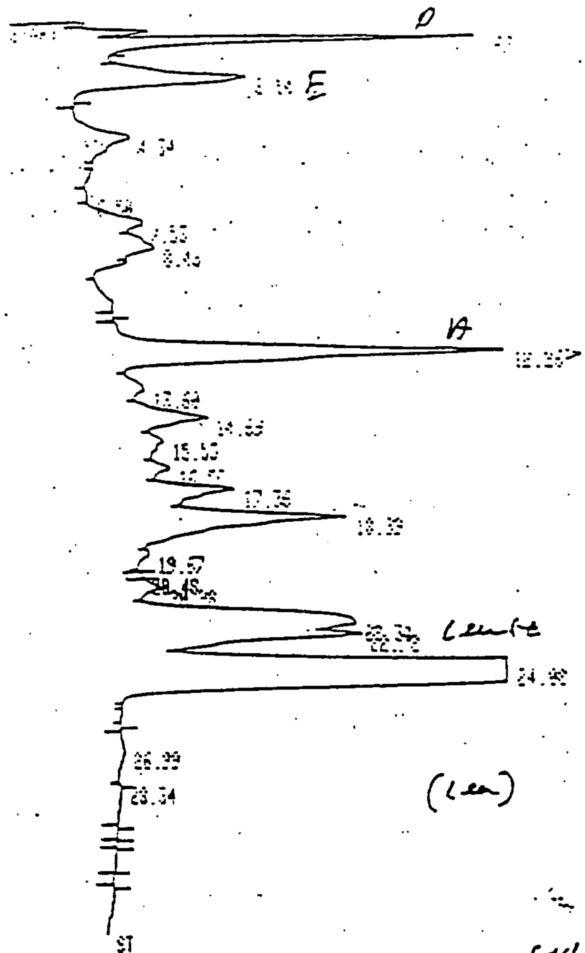
OCT/02/92 10:24:06

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.37	211870	PV	0.285	0.623
0.67	1493400	VB	0.214	4.406
2.13	1435200	SB	0.518	4.229
4.31	616270	PV	0.713	1.812
5.33	113550	VB	0.604	0.334
7.50	523310	BV	0.524	1.250
8.39	1113300	VP	0.914	3.274
12.45	3196200	BV	0.583	9.397
13.70	418310	VV	0.683	1.231
14.80	1026900	VV	0.602	3.196
15.59	436100	VV	0.472	1.283
16.52	586020	VV	0.566	1.723
17.27	1269000	VV	0.604	3.730
18.26	1967300	VV	0.518	5.786
18.75	638340	VV	0.391	1.877
19.56	259800	VV	0.546	0.764
20.30	161160	VV	0.396	0.474
20.77	369210	VV	0.556	1.086
21.93	1936400	VV	0.587	5.694
22.52	1485700	VV	0.443	4.368
23.88	1.4559E+07	VV	0.644	42.800

TOTAL AREA= 3.4011E+07
MUL FACTOR= 1.0000E+00

000232



ATT-4

cy17
LIFD α-factor
med.

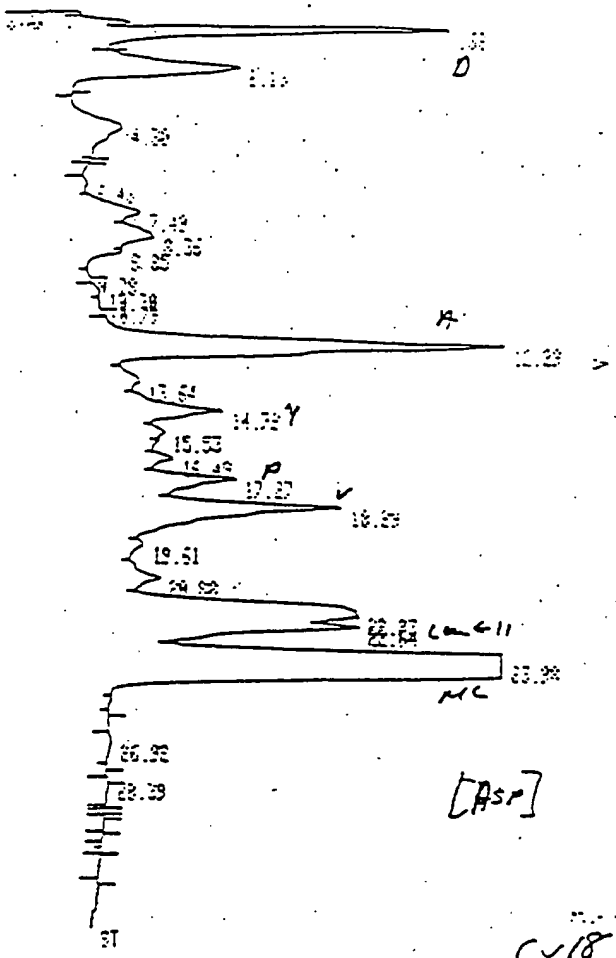
RUN # 951   OCT/02/92 11:42:08
ID 091

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.33	209070	PV	0.196	0.634
0.67	1422300	VB	0.223	4.310
2.14	1280800	BB	0.497	3.881
4.34	620340	PV	0.752	2.898
7.53	586600	VV	0.680	1.778
8.43	837690	VV	0.742	2.539
12.26	3211300	BV	0.479	9.731
13.60	293220	VV	0.669	0.889
14.69	899400	VV	0.611	2.726
15.55	551900	VV	0.327	1.673
16.56	413220	VV	0.559	1.267
17.36	1100000	VV	0.609	3.333
18.39	2268400	VV	0.624	6.874
19.67	106550	VP	0.436	0.323
20.98	125530	BV	0.343	0.388
22.31	2742700	VV	0.729	8.311
22.72	1778500	VV	0.459	5.390
24.08	1.4470E+07	VP	0.646	43.872

TOTAL AREA= 3.3000E+07
MUL FACTOR= 1.0000E+00

000233



ATT-4

Cy18
UFD-α-factor
med.

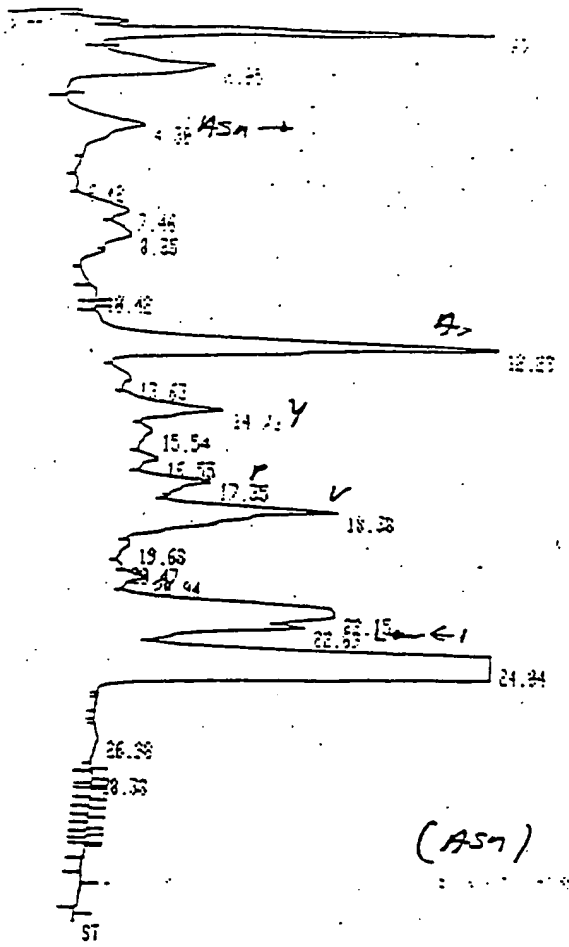
RUN # 352 OCT/02/92 13:00:10
ID 091

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.37	164770	PV	0.225	0.454
0.68 D	1712900	VB	0.295	4.719/143
2.13	1374800	BB	0.563	3.788
4.30	566300	PV	0.840	1.560
7.49	544920	VV	0.692	1.501
8.36	806980	VV	0.715	2.223
8.65	188540	VP	0.311	0.519
12.29	3449400	BV	0.510	9.503
13.64	401170	VV	0.723	1.105
14.72	1246800	VV	0.654	3.433
15.53	492990	VV	0.587	1.358
16.49	644350	VV	0.588	1.775
17.27	1327600	VV	0.629	3.658
18.29	2628500	VV	0.680	7.219
19.61	379820	VV	0.635	1.046
28.88	658800	VV	0.747	1.813
22.23	3267500	VV	0.792	9.002
22.64	1905300	VV	0.463	5.249
23.98	1.4547E+07	VV	0.642	40.075

TOTAL AREA= 3.6299E+07
MUL FACTOR= 1.0000E+00

G00234



(ASn)

ATT=4

Cy19
Lipoid Prot

RUN # 953
ID 331

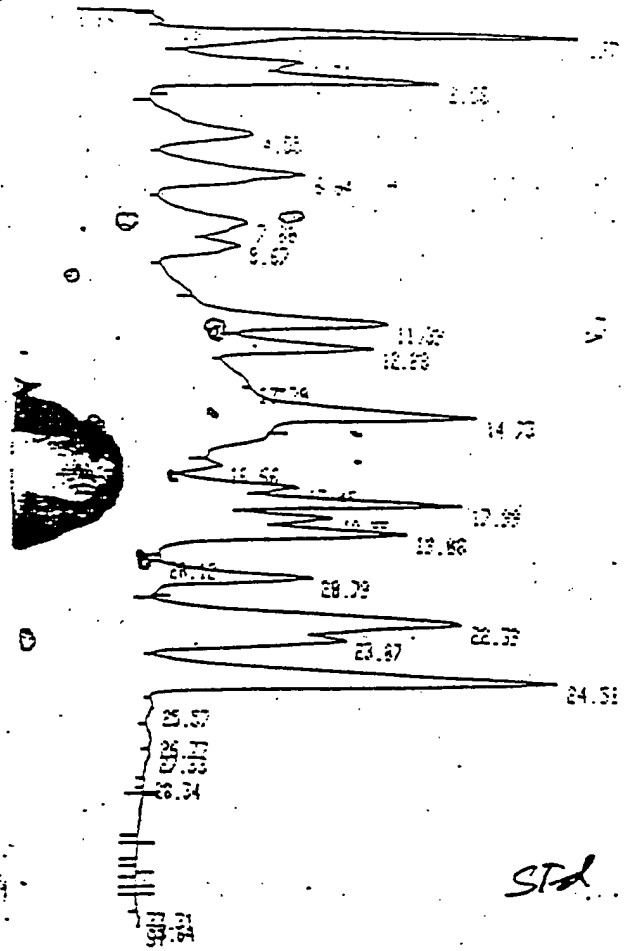
OCT/02/82 14:16:11

AREA%

RT.	AREA	TYPE	AR/HT	AREA%
0.37	170600	PV	0.209	0.464
0.67	1595600	VB	0.248	4.335
2.85	1254600	BB	0.538	3.409
4.38N	1063100	PV	0.822	2.888 /15
7.46	564050	VV	0.615	1.533
8.35	699760	VV	0.763	1.901
12.28	3525300	BV	0.499	9.578
13.63	503530	VV	0.777	1.368
14.71	1424200	VV	0.659	3.870
15.54	884540	VV	0.866	2.403
16.55	718110	VV	0.639	1.951
17.35	1134000	VV	0.577	3.081
18.38	2184800	VV	0.543	5.936
19.68	440210	VV	0.668	1.196
20.47	138200	VV	0.253	0.376
20.94	545650	VV	0.595	1.483
22.15	3386800	VV	0.824	9.982
22.69	1582400	VV	0.452	4.299
24.84	1.4954E+07	VV	0.653	40.627
26.98	118430	VV	0.889	0.322

TOTAL AREA= 3.6807E+07
MUL FACTOR= 1.0000E+00

000235



STD

RUN # 934
ID 891

OCT-01-92 13:35:41

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.77	4143888	PB	0.291	5.856
1.91	2083868	BV	0.465	3.315
2.58	3333388	VB	0.388	5.247
3.55	2555388	BV	0.746	4.229
6.84	3385888	VV	0.678	5.683
7.68	2453968	VV	0.795	4.869
8.67	1581288	VB	0.542	2.484
11.39	3886288	BV	0.435	4.975
12.28	2191188	VP	0.421	3.626
13.78	227188	PV	0.552	0.376
14.73	3581188	VB	0.486	5.794
16.66	247228	BP	0.298	0.489
17.45	1482588	PV	0.372	2.453
17.99	3632388	VV	0.382	6.011
18.55	2122188	VV	0.394	3.512
19.86	3627886	VP	0.451	6.883
20.79	2278288	VB	0.439	3.770
22.39	7064886	BV	0.689	11.691
23.87	2949988	[illegible]	0.455	4.882
24.51	8167988	[illegible]	0.688	13.516
25.57	119898	[illegible]	0.558	0.198
26.77	128888	[illegible]	0.629	0.213
27.33	183228	VV	0.564	0.303

TOTAL AREA= 6.8431E+07
MUL FACTOR= 1.0000E-06

000236